The University of Sydney
Centre for Veterinary Education
Professional Development Leaders

CANINE MEDICINE

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The Publisher

Speaker Profile

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Graham Swinney graduated from the University of Sydney in 1987. He then took up a position at Massey University Dept of Veterinary Clinical Sciences as the Assistant Lecturer Small Animal Medicine. From there he spent 4 years at University of Queensland as the Resident in Small Animal Medicine.

In 1993 Graham returned to the University of Sydney as Registrar and subsequently Senior Registrar Small Animal Medicine gaining his FACVSc Canine Medicine.

In 1999 Graham took up the position as Clinical Assistant Professor Small Animal Internal Medicine Department of Veterinary Clinical Sciences, Washington State University.

In 2005 he returned to Sydney and is now in private practice at the Veterinary Specialist Centre in North Ryde.

He is interested in all facets of canine medicine, especially endocrinology and oncology!

Program

SUNDAY 9 NOVEMBER 2008

9.00am - 11.00am	**Getting the most out of routine clinical pathology** A blood count, biochemical profile and urinalysis are tests that are a routine part of our day to day lives in clinical practice. How often do you just read the comments at the end, or look for the results marked as being out of the reference range? In this presentation we will look at some of these common tests to help you try to get more from these routine tests. We will also look at some less routine, but also very useful tests that are available to you today.
11.00am - 11.30am	***Morning Tea***
11.30am - 12.30pm	**A Rational Approach to Fever** How often are you presented with a patient with the complaint of being 'just not right', and the only thing on physical examination is a fever. Using case studies we will discuss a rational approach to the patient with a fever of unknown origin, including the basic tests that you will use in most cases, through to a look at tests to pursue some of the less common causes.
12.30pm - 1.30pm	***Lunch***
1.30pm - 3.30pm	**Approach to Anaemia** Anaemia is a common problem in your clinical practice. Based around case material we will discuss the approach to the anaemic patient. The approach to both regenerative and non regenerative
3.30pm - 4.00pm	***Afternoon Tea***
4.00pm	**Finish**

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Chapter 1

Maximising the Use of Your Clinical Pathology Laboratory

Dr Graham Swinney

Making the most of clinical pathology involves a number of different aspects, not limited to appropriate sample collection, the selection of appropriate tests, the interpretation of the test results, and recognising what can be done effectively ‘in house’, and what should be submitted to an external laboratory.

The plan in this presentation is to cover some aspects of a number of these issues, but it will not be all encompassing.

APPROPRIATE SAMPLE COLLECTION

This topic includes both what type of sample should be collected, and how to appropriately collect the sample you have decided on.

Cytological Samples

The quality of the material submitted to a clinical pathologist (or even if you are examining the cytology yourself in house), is going to influence how the cytology is interpreted. Basically, if a clinical pathologist receives a poor quality sample, they will have difficulty in providing you an accurate interpretation.

When aspirating cutaneous nodules, lymph nodes, or other palpable masses, care must be taken not to aspirate too vigorously. If using a needle and syringe together, active aspiration should be performed gently and aspiration stopped when material is seen in the hub of the needle (unless fluid is being aspirated). I tend to not have a syringe on the needle. Rather, I stabilise the lesion to be aspirated with one hand, and just insert the needle in multiple directions through whatever is being aspirated (if size allows this). Then I use an air-filled syringe to expel the aspirated material onto a slide. Some tissues tend to be bloody no matter how you aspirate them – thyroid neoplasms in dogs are a good example. Some harder lesions may require some degree of active aspiration to obtain a sample.

When it comes to making the smears of aspirated material, the cardinal rule is to be gentle. Some cells (such as lymphocytes) tend to be fragile, and can easily be ruptured. Most cytologists prefer to look at intact cells, rather than separate nuclei and cytoplasm. Using a second slide, gently place a second slide on top of the aspirated material, and gently pull the slides apart. Air-dry the slides immediately.

Joint Taps

To avoid blood contamination of arthrocentesis samples, I disconnect the syringe from the needle before I remove the needle from the joint. With the viscid nature of the joint fluid, even when any negative pressure has been released from the syringe, blood contamination may occur as the needle is withdrawn through the skin.

Joint fluid can be difficult to culture at the best of times. Samples to be cultured should be submitted as soon as possible. The yield from joint fluid cultures can be maximised by placing the fluid obtained in a blood culture bottle, rather than in a plain sterile tube. Don’t forget to request *Mycoplasmal* cultures as well.

Bone lesions

If suspicious bone lesions are detected on radiography, there may be a question of whether it could be neoplastic or infectious in nature. Often a bone biopsy is what is undertaken at this juncture. Some potential disadvantages of collecting a biopsy include the requirement for sedation or general anaesthesia, the potential for non-diagnostic samples (the dreaded reactive bone diagnosis), and a risk of pathologic fracture. Whilst they will not always be diagnostic, fine needle aspirate samples of the bone may be helpful. Sometimes what is not found may be helpful – if minimal inflammatory cells are found, it increases the index of suspicion of a neoplastic process.

Body Cavity Effusions

When there is a suspicion of a pleural effusion, it is an option to attempt a thoracocentesis before radiographs are taken. This approach has a number of advantages. The thoracocentesis can be both diagnostic and therapeutic (draining some fluid can be very helpful in a dyspnoeic patient), and radiographs taken with a significant volume of pleural effusion will reveal only the effusion (and may present some risk to the patient if they have significant respiratory distress).

For smaller dogs, the equipment required is a syringe, a three-way tap/stopcock, and a butterfly needle. Connect the equipment in line, and then gently aspirate as the needle is inserted in the intercostal space – often in the sixth to eighth intercostal spaces, about one third of the way between the sternum and spine. Once fluid is obtained, place some into an EDTA tube, and some into sterile serum tubes, so fluid is available for cytologic and microbiologic examination. In larger dogs, where the butterfly needles are not long enough to gain access to the pleural space, it is best to use the syringe, three-way tap/stopcock, a fluid administration extension set, and a needle or a catheter.

For performing an abdominocentesis, the butterfly needle and syringe, or a catheter, extension set and syringe are options.

Respiratory/Pulmonary Cytology

Briefly addressing the upper respiratory tract - nasal cytology is not frequently that helpful in the aetiologic diagnosis of animals with nasal disease. It is normal to find bacteria, and in some cases even fungal organisms may represent an incidental finding. On the same level, cultures of nasal discharge are rarely useful.

There are a number of different options for the collection of material for airway/pulmonary cytology. These include transtracheal aspirates/wash, bronchial wash, bronchoalveolar lavage, and pulmonary fine needle aspiration.

Transtracheal aspirates have the advantage of being possible without general anaesthesia, and they also avoid oropharyngeal contamination, making the samples useful for microbiologic analysis. They can be performed using specifically designed kits, a through the needle (long) intravenous catheter, or a large needle and a soft feeding tube. Disadvantages include the fact that only the upper respiratory tract is being sampled, and there have been cases where the catheter has been cut off, necessitating removal from the trachea.

Bronchial washes or bronchoalveolar lavage are commonly performed techniques. The main difference between the two is that with a bronchoalveolar lavage, one particular area of the pulmonary tree is being sampled – often achieved by wedging an endoscope in an airway, but this can also attained by wedging a sampling catheter into an airway. In a bronchial wash, the patient is lightly anaesthetised and intubated. In small dogs, a Christmas tree adaptor can be placed into the end of the endotracheal tube, and the wash is performed via the cuffed tube. In larger patients a soft catheter (such as a nasogastric feeding tube) is inserted through the endotracheal tube as far as it will comfortably pass. It is advantageous if the animal is under a very light plane of anaesthesia, so they will cough when the wash is performed. This technique can obtain deeper samples than a transtracheal wash, and the samples are more voluminous. Because there is usually at least some degree of oropharyngeal contamination, culture results must be interpreted with this in mind.

Pulmonary fine needle aspirates are really the simplest of the techniques to obtain pulmonary cytology – but likely the one that scares a lot of practitioners the most. This technique is very useful for nodules or masses (as they rarely exfoliate cells with the wash techniques described above), but is also useful for diffuse pathology. In many patients aspirates can be obtained with either light or no sedation. A 23 to 25 gauge needle, or spinal needle if more length is needed can be used, with a syringe attached. The skin should be clipped and aseptically prepared. The needle is inserted into the area of interest, and gentle aspiration is applied. Repeated redirection is not performed, such as with a cutaneous or lymph node aspirate. If a mass lesion is to be aspirated, and it is adjacent the thoracic wall, ultrasound guidance can be utilised. In other cases, fluoroscopic guidance can be used. Patients with bleeding disorders are not good candidates. The main risks for the procedure include pneumothorax or haemorrhage. The samples obtained are also very suitable for microbiologic analysis.

Bone Marrow Cytology

Bone marrow aspirates may be indicated in cases of non-regenerative anaemia, thrombocytopaenia, leucopaenia, or to help diagnose or stage some neoplastic diseases. There are a number of sites that can be employed, including the proximal humerus, wing of the ilium, or the femur. In cats, the femur is often the preferred site.

Materials required include bone marrow aspirate needles (that have a stylet), or a Jam Shidi bone biopsy needle, some sterile anticoagulant, and a watch glass or dish in which to place the sample. The needle should be flushed with EDTA. After insertion of the needle (with the stylet in place), it should be firmly seated in the bone. Once the stylet is removed, a syringe is attached, and aspiration performed. Once marrow enters the syringe, stop aspirating, and squirt the marrow into the watch glass that also contains EDTA, and mix. Smears can then be made of the marrow, and marrow material left over can be placed into an EDTA blood tube for submission. If using a Jam Shidi needle, and a poor sample is obtained upon aspiration, the needle can be advanced with the stylet out, and a bone marrow core sample can be collected. This can be rolled on a slide for cytologic examination, and can be placed in formalin for histopathologic examination.

Other Cytologies

In some cases, intra-abdominal pathology may be suspected based upon clinical examination – such as palpable hepatomegaly, splenomegaly or renomegaly. If these organs are to be aspirated, it is always preferable to obtain these samples with ultrasound guidance. However in some cases, such as constraints placed by a client, this may not always be possible. As long as the client is aware of risks, such as haemorrhage, or seeding neoplastic cells or infectious agents, these organs can be aspirated blindly.

TEST SELECTION/INTERPRETATION/IN-HOUSE VS SEND-OUT

Initially, we will address the major categories of haematology, biochemical tests, and urinalysis.

Haematology

At the most basic level, many practitioners will check a packed cell volume patients, including those with anaemia, a history of trauma, or as a routine pre-anaesthetic check. It is always very important to check a total protein concurrently. A fall in the total protein is suggestive of blood loss. This can even be important if monitoring a patient where the cause of anaemia is known – a patient being immunosuppressed for a haemolytic cause of anaemia that has a plateau or fall in PCV, that also has a falling total protein may have developed gastrointestinal ulceration, with ensuing blood loss.

With the advent of many in-house haematologic instruments, many practitioners now perform many of the routine blood count analyses in-house. Obviously there are numerous advantages, including financial, and not the least rapid turn around times for results. There are some important factors to consider however when using such instruments. These instruments require routine maintenance, quality control and calibration. For example it is recommended that two different control samples should be run during an eight-hour period of patient testing, or any time reagents are changed. The instrument should be calibrated after a major service, if indicated be quality control data, if changing reagent type, or at least every six months. If these are not performed, the results of the instruments may be called into question.

The automated in house analysers do not provide a review of a blood smear. In some cases this will not be a problem. In other cases this will be a huge issue, as it will mean you are missing vital information. Many of these machines will separate granulocytes and lymphocytes/monocytes. However different granulocytes will mean different things. Take for example a patient with fever and back pain. An in house blood count revealed a total WBC count of 14,000/uL, with 12,000/uL granulocytes and 2,000/uL lymphocytes/monocytes. Then a CBC was repeated with a smear review, with similar total numbers. However a differential count revealed over half of the neutrophils were bands, and all the neutrophils exhibited significant toxic changes. This definitely could change the interpretation of the CBC. The dog likely has an infection. In other cases the author has been involved; patients have been referred because of persistent leucocytosis. The only diagnostic test performed at the referral hospital has been a CBC with smear review, which has revealed the presence of a leukaemia. Smear review in a patient with thrombocytopaenia can also be helpful – the presence of large platelets may suggest a regenerative response. A smear review in an anaemic patient that reveals many acanthocytes could increase suspicion of a disease process such as haemangiosarcoma. Anisocytosis and polychromasia are generally suggestive of a regenerative response.

Therefore it is strongly recommended to include a review of a blood smear as part of any in-house CBC. Obviously if any unusual cell morphology is detected, it is worthwhile to send a blood sample to a laboratory with a specialist clinical pathologist for review.

In anaemic patients, review of the red cell indicies is very important. Ideally a reticulocyte count will also be evaluated, but in the absence of this, the indicies may give some clues as to the nature of the anaemia. In a typical regenerative anaemia, there will be a macrocytosis (elevated MCV) and hypochromasia (reduced MCHC). It is important to remember that the absence of macrocytosis does not rule out the possibility of a regenerative response. The presence of regeneration, as evidenced by increased reticulocytes, in the face of a normal MCV, is suggestive of the presence of a disease often associated with microcytosis. Most commonly this will be some cause of chronic blood loss, or a bleeding disorder.

The presence of nucleated red blood cells on a smear does not always mean regeneration. They may be part of a more intense regenerative response in some situations, but they can also occur with bone marrow pathology, splenic disease, lead intoxication, and may also be breed associated (Miniature Schnauzers).

There are some more common causes of microcytosis in small animal practice. These include iron deficiency anaemia, most commonly associated with chronic blood loss. In such cases the gastrointestinal tract is the most common culprit, even in the absence of obvious clinical signs such as melaena. Other possible sources of chronic blood loss are the respiratory and urinary tracts. Portosystemic shunts in dogs are often associated with microcytosis, probably related to iron redistribution rather than iron deficiency. More specifically, there are some breed related cases of microcytosis, notably the Japanese Akita, and the Shiba Inu.

Remember, in some cases a normal leucogram may be suspicious. A good example could be a patient with hypoadrenocorticism, which may have non-specific clinical signs. Not all hypoadrenocorticoid patients will have mineralocorticoid deficiency, which means that the sodium and potassium may be normal. In a patient that is ill, and there is no stress leucogram, the possibility of hypoadrenocorticism manifest only by a glucocorticoid deficiency should be considered.

Biochemical Profile

To begin with, many of the comments made about in-house haematologic instruments apply to in-house biochemical analysers. Routine maintenance, quality control and calibration are paramount to obtaining accurate results.

Here are a few things that can be valuable when interpreting the results of biochemical profiles.

- The liver enzymes, ALT (alanine aminotransferase) and ALP (alkaline phosphatase) are not indicators of hepatic dysfunction. The ALT is an indicator of hepatocellular damage, and the ALP is an indicator of cholestasis, bony damage/remodelling, or it can be induced by glucocorticoids. On a routine biochemical profile in a dog, potential indicators of hepatic dysfunction would include hypoglycaemia, hypoalbuminaemia, hypocholesterolaemia, and a reduced blood urea. .

- An elevation in blood urea in the face of a normal creatinine and phosphorus may be an indicator of gastrointestinal haemorrhage.

- When considering causes of hypoalbuminaemia, there are a significant number of causes including situations of reduced production (hepatic dysfunction, or cases where albumin production is reduced because it is a negative acute phase reactant protein), or increased loss (renal, gastrointestinal, haemorrhage, cutaneous, vasculitis). If the globulin concentration is normal, all remain possibilities. If there is a panhypoproteinaemia, gastrointestinal loss or haemorrhage represent the most likely causes.

- In the face of an animal with hyperkalaemia, there are a number of potential causes, including hypoadrenocorticism, acute renal disease, urinary retention (urethral obstruction, urinary tract rupture), gastrointestinal disease (including trichuriasis), pleural effusions, or incorrect anticoagulant use (such as potassium EDTA). If the sodium is normal, it is worth looking at your haemogram. In patients with marked thrombocytosis, there can be an artifactual hyperkalaemia. This can be ruled out be checking a plasma potassium concentration. Also note that if the patient is a Japanese Akita or Shiba Inu, that they have a high erythrocyte potassium concentration, so haemolysis may result in an artifactual hyperkalaemia.

- Not all patients with elevated amylase and lipase have pancreatic disease. Serum amylase and lipase have long been used to try and confirm the diagnosis of canine pancreatitis. However both have limited sensitivity and specificity. Whilst the pancreas synthesises lipase, so do other tissues and the catalytic assay utilised does not differentiate the source of lipase. This is evidenced by the fact that patients with exocrine pancreatic insufficiency may have a normal lipase. Lipase elevations may occur with renal failure, glomerulonephritis, hepatic disease, intestinal disease, the administration of glucocorticoids (especially dexamethasone), or heat stress. The information for serum amylase is very similar, with many non pancreatic sources. It is important to note that about 50% of patients with an elevated serum amylase, lipase, or both do not have pancreatitis.

- The serum trypsinogen-like immunoreactivity test (TLI) detects trypsinogen and some trypsin in the circulation, and is a species specific test. The test has proven to be very specific for the diagnosis of EPI in dogs and cats. It was found that dogs with experimentally pancreatitis had elevated TLI levels. Some dogs with spontaneous pancreatitis will have an elevated TLI level, but this may be less than 40% of patients. Sensitivity and specificity have been reported as 65% and 33% respectively, limiting the diagnostic value.

- As discussed previously, one of the limitations of the catalytic assay for lipase is that it does not differentiate the many sources of lipase in the body. The different lipases do have different molecular structures. The gastrointestinal laboratory at Texas A and M University purified classical pancreatic lipase (cPL) from dog pancreas, and developed antisera against this in rabbits. Further testing demonstrated that the only cell type staining positively for cPL was the pancreatic acinar cell, and a reference range was developed using 74 clinically healthy dogs. A radiommunoassay and ELISA were developed and validated, with the ELISA commercially available. The cPLI levels were demonstrated to be reduced in dogs with EPI. The cPLI was evaluated in dogs with chronic renal failure, and it was found that the levels were within reference ranges, and promising results were obtained in patients with gastritis. The levels are not affected by the administration of prednisolone. Another study compared the results of various diagnostic tests in cases of biopsy proven pancreatitis. The sensitivity was shown to be over 80%. Further evaluation may be necessary in patients with mild or chronic disease.
- The serum sodium concentration is not only a reflection of the sodium level, but also of water balance. So in some situations, such as congestive heart failure or hepatic dysfunction, there may be hyponatraemia.
- When assessing the serum calcium level, it is important to assess the phosphorus level as well. If a patient is hypercalcaemic, but has hypophosphataemia, the patient likely has hypercalcaemia of malignancy, or primary hyperparathyroidism, due to the influence of PTH or PTHrP. The hypophosphataemia may be blunted, and the patient may become hyperphosphataemic as they go into renal failure.
- If the patient is hypercalcaemic and azotaemic, in the vast majority of cases the hypercalcaemia caused the renal failure, rather than the renal failure causing the hypercalcaemia.
- Ionised calcium concentrations are very valuable if you can get access to them. It removes the effect of albumin concentrations, and gives an indication of the functional calcium level.
- Note that the use of heparin as an anticoagulant will reduce the measured iCa concentration.
- Sepsis can be associated with significant ionised hypocalcaemia.

- In patients with a high anion gap metabolic acidosis (can be calculated by adding sodium and potassium levels, and subtracting chloride and bicarbonate levels), causes include ethylene glycol toxicity, renal failure, lactic acidosis, diabetic ketoacidosis, and salicylate toxicity.
- In a patient with a high bicarbonate (metabolic alkalosis), and a low chloride, think gastric outflow obstruction or possibly a motility disorder

Urinalysis

The urinalysis often represents an under-utilised part of a diagnostic work up, but is certainly a test that can be performed 'in-house'. A biochemical profile cannot be interpreted without the results of the UA. Ideally, the urine sample should be collected at the same time as the blood, and certainly before any therapy. A complete UA involves some physical assessment, a chemical assessment, and finally an examination of the sediment. The ideal way to collect the sample is via cystocentesis. However there are some relative contraindications to performing a blind cystocentesis, including a bleeding disorder, a dog with suspect pyometra, or a peritoneal effusion.

The urine should be evaluated for colour and turbidity, and a specific gravity checked. The chemical analysis can be performed using test strips. If using the human strips, the nitrate, urobilinogen and leucocyte tests are not reliable. If a positive protein reaction is obtained, it can be confirmed with the SSA test. The urine should then be centrifuged, and an examination performed on the sediment. The strips are most sensitive to albumin, but an active sediment can cause false positives. The SSA test will also detect globulins and Bence Jones proteins. Proteinuric patients can be further evaluated using a protein: creatinine ratio to better assess the severity of the proteinuria. However a sediment examination should be performed first, as active urine sediments may influence the results.

Let us consider some aspects of a biochemical profile that require the UA to interpret, or are enhanced by the UA.

- Azotaemia cannot be classified as pre-renal, renal, or post-renal without a UA.
- An acutely azotemic patient with calcium oxalate monohydrate crystals in the urine has likely been exposed to ethylene glycol.
- If a patient is hypoalbuminaemic, the UA is important to rule in or out renal loss as a potential cause.

- In a hyperglycaemic patient, it is important to confirm glucosuria.
- Glucosuria in the absence of hyperglycaemia may indicate a tubular dysfunction, such a primary renal glucosuria or Fanconi's syndrome. It is important to note that both cephalexin and enrofloxacin can cause false positive or false negative urine glucose results.
- The presence of ketonuria in the face of hyperglycaemia is diagnostic of diabetic ketoacidosis.
- If amylase and lipase are elevated, but the urine is concentrated, renal dysfunction is unlikely.
- Bilirubinuria will precede hyperbilirubinaemia.
- Alkaline urine in the face of metabolic acidosis may suggest a renal tubular acidosis.
- Patients with persistently low urine specific gravities are likely PU/PD, even if not noted by the owner.

Other Miscellaneous Tests

Endocrine Testing

- When interpreting the results of a PTH assay in a hypercalcaemic patient, evaluate it in light of the ionised calcium concentration. PTH concentrations will vary with small changes in the ionised calcium concentration. Therefore, in the face of elevated ionised calcium, even a PTH level within the reference range is abnormal – it should be low if the parathyroid glands were functioning normally.
- When monitoring a diabetic patient, glucose curves are required periodically. Spot checks can be misleading.
- Thyroid panels are our best way to diagnose hypothyroid dogs. Look for low total T4, elevated TSH, and low free T4 (measured by equilibrium dialysis).
- When an animal is 'sick' is not the ideal time to test for hyperadrenocorticism. Whilst the ACTH stimulation test is more specific than the low dose dexamethasone suppression test, both can be affected by non-adrenal illness. Remember that hyperadrenocorticoid patients are rarely 'sick', so if they are, there is likely some other concurrent illness.

Body Cavity Effusions

- In the initial assessment of a body cavity effusion, a lot can be gained from checking a total protein on a refractometer and looking at a smear.
- A relatively acellular effusion, with a very low total protein (< 15 g/L) is likely a pure transudate. Therefore an investigation for causes of hypoalbuminaemia will likely be indicated.
- Moderate protein levels and cellularity are suggestive of a modified transudate – so heart failure, some hepatic diseases (sinusoidal or post-sinusoidal), neoplasia become more likely.
- High cells and protein are suggestive of an exudate. If organisms are seen on a smear, it means aggressive therapy, and diagnostics to determine the source of the organisms are indicated.
- When treating a pyothorax, periodically make smears of the drained material. If a gram stain shows a lack of organisms, the therapy chosen is likely effective.

Blood Cultures

- Don’t be shy about doing these on patients with fevers of unknown origin, discospondylitis, endocarditis, polyarthritis etc

Blood Gases

If you have access to equipment that can measure blood gases, there are a number of indications to assess an arterial blood sample.

These include animals with upper respiratory tract disease, lower respiratory tract disease, neurologic disease affecting ventilation, pleural space disease, thoracic wall disease, pulmonary vascular disease, or some systemic metabolic diseases.

Assessing the PaC02 is the only way to assess a patient’s ventilatory capacity.

Assessing the Pa02 is an objective way to assess gas exchange.

Diagnosis of DIC

- There is no way to definitively rule in or out DIC based on laboratory testing, but the laboratory tests can provide support for a clinical suspicion of the disease process.
- Useful laboratory tests include
    - Platelet counts – most DIC patients will become thrombocytopaenic.
    - Prothrombin time, Partial Thromboplastin time – both will likely become prolonged.
    - D-Dimers will typically be elevated.
    - Antithrombin III levels – they will likely be low, but it is not clinically useful due to the long turn around time for the test.
    - Blood smears may show signs of a microangiopathy.

INTERPRETATION OF TEST RESULTS IN LIGHT OF THE PATIENT

The final point will be to always relate any test results back to the patient being evaluated. The question of “Do these results explain what is being seen clinically” must be answered. At times results may be obtained that indicate a problem, but not one that would explain why the patient was presented, and treating the laboratory problem alone will not result in the resolution of the patient’s disease. On the other hand, if you have a strong clinical suspicion of a disease, but the laboratory tests do not support these suspicions, retesting may be warranted.

Question 1

When considering a patient with hyperkalaemia, which of the following can be a cause of an artifactual hyperkalaemia?

A. Neutrophilia
B. Erythrocytosis
C. Metabolic Alkalosis
D. Marked Thrombocytosis

Question 2

In the consideration of an anaemic patient, which of the following statements is incorrect?

A. In an acutely anaemic patient, a reduced PCV and TP are most suggestive of blood loss
B. A reticulocyte count is the most accurate way to assess regeneration
C. An anaemic patient with an elevated nucleated red blood cell count has a regenerative anaemia
D. A normal MCV is not always diagnostic of a non-regenerative anaemia

Question 3

When evaluating fluid obtained from the thoracic cavity, which of the following is incorrect?

A. A fluid with a nucleated cell count of 2,100/uL and total protein of 25 g/L is likely the result of hypoalbuminaemia
B. A fluid with a cell count of 2,500/uL and a total protein of 30 g/L is consistent with a modified transudate
C. The presence of micro-organisms would be classified as a septic exudate
D. A fluid with a PCV of 6% would be classified as a haemorrhagic effusion

Question 4

In a patient with a total protein of 45 g/L, and an albumin of 10 g/L, which of the following statements is correct?

A. Hepatic disease will cause both hypoalbuminaemia and hypoglobulinaemia
B. Vasculitis could be a cause of the hypoalbuminaemia
C. Such a patient will likely have a protein losing enteropathy
D. The patient does not have a protein losing nephropathy

Question 5

In a patient with hypercalcaemia (both total and ionised), which of the following is incorrect?

A. If the PTH level is normal, hyperparathyroidism is excluded as a cause
B. If the PTH level is high, hyperparthyroidism is confirmed
C. If there is concurrent hypophosphataemia, malignancy or hyperparathyroidism are most likely
D. If there is concurrent hyperphosphataemia and azotaemia, malignancy or hyperparathyroidism cannot be excluded

Answers

1. D
2. C
3. A
4. B
5. A

NOTES:

NOTES:

Chapter 2

The Fever of Unknown Origin Patient – A How To Guide

Dr Graham Swinney

DEFINITIONS

Hyperthermia

The elevation of body temperature above the accepted reference range. It represents an imbalance in the normal equilibrium of heat balance, where heat is produced or stored in the body at a greater rate than heat loss via radiation, convection or evaporation.

Fever

Hyperthermic patients with a true fever (pyrexia) have an alteration in the 'set point' temperature. The thermoregulatory centre is located within the central nervous system in the anterior hypothalamus.

Patients with an elevated body temperature without a true fever have a normal set point temperature. There are peripheral and central thermoreceptors in the body that detect ambient and core temperatures. The information gathered is then transmitted to the thermoregulatory centre via the nervous system. If the body temperature is low the thermoregulatory centre will stimulate heat production via release of catecholamines and thyroxine, along with shivering, and increase heat conservation via vasoconstriction, piloerection, postural changes such as curling up, and actively seeking a warmer environment. If the body temperature is high, the animal will likely have vasodilation, panting, stretch out, and seek a cool environment.

HYPERTHERMIA

Hyperthermia may be the consequence of inadequate heat dissipation. This can be the result of a hot environment, such as an animal being locked in a hot car or room. There can be situations where dogs may exercise in a hot and/or humid environment. Sustained exercise can result in increased heat production because of the muscular activity, sometimes even in the absence of high ambient temperature or humidity. The heat accumulation may be exacerbated in dogs that are overweight. Patients with respiratory disease can also have impaired heat dissipation, and hyperthermia can be a presenting complaint for some patients with laryngeal paralysis.

Sympathetic stimulation as a result of agitation and nervous activity may result in an elevated body temperature in some patients undergoing physical examination.

There may be pathologic causes of increased muscle activity, such as hypocalcaemia with tremors, eclampsia, seizures, or metaldehyde toxicity. In malignant hyperthermia pharmacologic agents can induce a myopathy, with resultant muscle stiffness. Some inhalational agents (Halothane), local anaesthetics and muscle relaxants (succinyl choline) have been implicated. Other metabolic diseases that can be associated with hyperthermia include hyperthyroidism and phaeochromocytoma, both of which can be associated with an increased metabolic rate and vasoconstriction.

MECHANISM OF FEVER

There are many causes of a true fever, including infectious disease, non infectious inflammation (such as immune mediated disease), immune complexes, neoplastic diseases, tissue necrosis, and potentially some pharmacologic agents. All these processes can produce substances called exogenous pyrogens. These pyrogens may be the infectious agent itself, or products produced by the organisms. The exogenous pyrogens themselves tend to not directly affect the thermoregulatory centre.

These exogenous pyrogens then act on cells of the immune system, and stimulate the release of cytokines that act as endogenous pyrogens. Macrophages are the main source of endogenous pyrogens, but B and T lymphocytes, and neutrophils can also be a source. Interleukin 1 is considered to be the most important of these cytokines, but there are a number of others that play a role including tumour necrosis factors alpha and beta, interferon alpha, beta and gamma, and interleukins 6 and 8. There are some neoplastic cell lines that can directly produce some of these cytokines. These cytokines travel in the vascular system to the hypothalamus, where they bind to vascular endothelial cells. Here they stimulate the release of prostaglandins, mainly PGE 2 alpha, which then cause the set point temperature to elevate. The body will then both generate more and conserve heat, and the body temperature will rise.

AETIOLOGY OF TRUE FEVER IN DOGS

In human medicine fever of unknown origin is defined as a fever that has persisted for at least three weeks, with no cause determined via laboratory testing or radiographs. In veterinary medicine there is not a strict definition, and any fever where there is no cause apparent on history or physical examination may be considered a fever of unknown origin.

As discussed previously, the causes of fever can include infectious disease, non infectious inflammation, neoplasia, or tissue damage. Infectious disease typically represents the most common cause of fever in dogs, but there are numerous potential aetiologies. Immune mediated diseases likely represent the next most common cause. Immune complexes are potent exogenous pyrogens, which then cause release of endogenous pyrogens. Neoplastic diseases are less common as a cause. As discussed, they may directly release cytokines that can affect the hypothalamus, but they may also be associated with tissue necrosis or inflammation. Tissue trauma may also cause a moderate fever, which may be spontaneous, but may also occur in a post surgical setting. In both cases the fever does not reflect infection, and antimicrobials are not indicated.

The list of potential infectious diseases is extensive, and can be considered based on the organism, or the tissue affected. The infections are more commonly bacterial, but viral, fungal, protozoal, rickettsial or parasitic causes are also recognised. Specific diseases would include endocarditis, pyometra, prostatitis, pyelonephritis, polyarthritis, pyothorax, discospondylitis, meningitis, pneumonia, or abscesses. Non infectious causes of inflammation may include pancreatitis, immune mediated diseases such as polyarthritis or systemic lupus erythematosus, or Shar Pei fever. Neoplastic diseases that have been associated with fever include lymphosarcoma, or some myeloproliferative diseases. Fever has even been reported as an unusual clinical sign in some patients with portosystemic shunts.

APPROACH TO A FEVER PATIENT

In many cases in patients with fever of unknown origin, the cause will not be apparent after a history and physical examination. A logical, systematic approach will be required to try and reveal the cause of the illness. The initial testing should be directed at more common causes of fever, but in time testing for less common causes, or more invasive testing may be required.

Signalment

Whilst it may not appear that signalment alone would provide much useful information, it can provide some information as to the cause of the fever. For example there are animals of certain breeds (Cocker Spaniels, Old English Sheepdogs, Poodles), age (young to middle aged) and sex (female) that have a higher incidence of immune mediated diseases. Older patients may be at a higher risk of neoplasia. Certain breeds have particular diseases associations, such as Shar Peis with a fever syndrome. Male dogs have a higher incidence of infectious polyarthritis. Younger large breed dogs may be more commonly associated with suppurative meningitis, and Beagles can have a meningitis syndrome associated with arteritis.

Environment

There can be geographic associations with certain types of disease. This may be less prominent in Australia, but for example in North America this is much more important._Certain infectious diseases are geographical, including rickettsial diseases, fungal diseases, babesiosis and leishmaniasis. Exposure to non domestic vectors or stagnant water may increase the risk of exposure to leptospirosis. Hunting dogs or field trial dogs may be at increased risk of inhalation of foreign bodies, with the consequence of increased risk of pulmonary or pleural space infections.

History

The history has the potential to give some clue as to the cause of the fever. In some cases it may provide some localising signs such as a cough, pollakiuria, stranguria, exercise intolerance, vomiting, stiffness, or lameness. Any previous illnesses should be noted, along with any recent wounds, or procedures such as dental cleaning or surgery. Questions about the dog’s environment and travel history could prove important. Clients should be questioned about recent medications or biologics, as some may be associated with immune mediated disease, for example vaccinations, penicillins, and cephalosporins. The history is also an important part in the determination of whether a patient with an elevated temperature has a true fever or hyperthermia. A history of a hot environment, recent exercise, muscle tremors, seizures, or drug exposure may indicate hyperthermia is more likely.

Physical Examination

The physical examination will be the initial means to establish that an elevated temperature is present. Once this has been established, a thorough physical examination is indicated. Any physical examination abnormalities may help focus any investigation to be performed. Areas that deserve particular attention on the examination include an ophthalmic examination, as there may be retinal or uveal changes with systemic diseases (infectious, immune or neoplastic), palpation of joints and bones to assess for swelling or pain, careful cervical and spinal palpation for pain or stiffness, thorough cardiac and pulmonary auscultation for murmurs, crackles or wheezes, thoracic percussion to check for pleural fluid, abdominal palpation for masses, fluid or pain, and examination of the lymph nodes for enlargement (reactive, inflammatory or neoplastic). In all dogs a rectal examination is indicated, with particular attention given to the prostate in a male dog. The prostate should be evaluated for size, shape, symmetry, contour, texture, mobility and pain. Overall palpation of the trunk and limbs for any swelling or pain is also important. Depending on the history and general physical examination findings, a neurological examination may be indicated. It is important to note that if a fever develops in a hospitalised patient, or a fever persists, serial physical examinations are indicated. If the patient has a catheter in place, always check the catheter site, as catheter induced phlebitis may be a source of infection and bacteraemia, especially in an immunocompromised patient

At this stage, depending on the duration and severity of clinical signs, and the client's willingness to pursue an investigation further, there is the choice of further investigation, or a therapeutic trial with antimicrobial medication. If the clinical signs are mild and of short duration this may be a viable option. If the antimicrobial trial was unsuccessful, there may be another trial with another antimicrobial or anti-inflammatories. However if the signs are severe, prolonged, or non responsive to an appropriate therapeutic trial, further investigation is indicated.

Further Investigation

Obviously if there are any suggestions based upon the history and physical examination, specific diagnostic tests may be indicated. If not, there is some common testing that should be considered.

Initial Diagnostic Testing

Haematology

A complete blood count is an important initial diagnostic test. The evaluation of red cell, white cells and platelets is essential. Anaemia may occur with chronic inflammatory disease, immune mediated disease including haemolytic anaemia or systemic lupus erythematosus, or some neoplastic diseases (including lymphosarcoma or myeloproliferative disease). The presence of strong regeneration, macrocytosis, and spherocytosis would suggest haemolysis as the cause of the anaemia. Erythrocytosis may be the consequence of pulmonary disease that could also potentially be associated with fever. Thrombocytosis can be associated with inflammatory diseases. Thrombocytopaenia can be associated with immune mediated diseases, but can also be the consequence of infectious diseases (rickettsial and ehrlichial diseases, adenovirus) or immune mediated diseases that cause vasculitis.

The leucogram may be suggestive of inflammatory disease if there is neutrophilia or monocytosis, both of which can also be associated with a stress leucogram. The presence of a left shift makes inflammatory disease more likely. An eosinophilia can be the consequence of a hypersensitivity reaction, infections with organisms with a close host-parasite relationship such as fungi, mucosal inflammation, or some neoplastic conditions. The use of in-house haematology units provides rapid results, but has some limitations. The automated in house analysers do not provide a review of a blood smear. In some cases this will not be of any consequence, as the cell morphology may be normal. In other cases this will be a huge issue, as it will mean you are missing vital information. Many of these machines will separate granulocytes and lymphocytes/monocytes. However different granulocytes will mean different things

A real case example was a dog presented with hind limb weakness and back pain. The referring veterinarian had performed an in-house haemogram, with a total WBC count of 14,000/uL, with 12,000/uL granulocytes and 2,000/uL lymphocytes/monocytes. The CBC was repeated upon admission, with similar total numbers. However a differential count revealed over half of the neutrophils were bands, and all the neutrophils exhibited significant toxic changes. This without doubt changed the interpretation of the CBC. The dog had a huge paraspinal abscess. In other cases the author has been involved; patients have been referred because of persistent leucocytosis. The only diagnostic test performed at the referral hospital has been a CBC with smear review, which has revealed the presence of a leukaemia. Smear review in a patient with thrombocytopaenia can also be helpful – the presence of large platelets may suggest a regenerative response. A smear review in an anaemic patient that reveals many acanthocytes could increase suspicion of a disease process such as haemangiosarcoma. Smear review may give indications of a regenerative anaemia.

Biochemistry

Whilst it may not be the most sensitive or specific test, a biochemical profile is indicated to help try and localise potential pathology causing a fever. For example the presence of azotaemia and fever raises the possibility of pyelonephritis, or elevated liver enzymes could suggest hepatic pathology. Whilst they have limited sensitivity and specificity, amylase and lipase levels can be used as a screening test for pancreatitis. Elevated creatine kinase levels could suggest muscle damage that may be related to immune mediated disease, infectious agents, trauma, muscle necrosis, or toxic causes.

Urinalysis

The diagnostic value of a urinalysis cannot be overemphasised for any sick patient. The specific gravity will be necessary to evaluate any changes in the renal values on a biochemical profile. In some animals with pyelonephritis there may be some loss of concentrating ability. The chemical analysis may show proteinuria, which could be pre-renal, such as seen with paraproteinaemias, or post renal that may be seen with cystitis or lower urinary tract haemorrhage. Renal proteinuria may be the consequence of tubular or glomerular disease. Glomerular protein loss may be the consequence of systemic disease that could be infectious, inflammatory, immune mediated or neoplastic. The presence of pigment in the urine (colour that does not clear with centrifugation) may be indicative of haemolysis if haemoglobin, or significant muscle damage if myoglobin, both of which may be associated with fever. The sediment analysis may show pyuria suggestive of urinary tract inflammation. Bacteriuria, in an appropriately collected sample, is an indication of urinary tract sepsis. It is important to remember that there are some diseases where in the face of urinary tract infection there may be no indications on sediment examination – namely diabetes mellitus and hyperadrenocorticism. Fungal hyphae may be detected in the urine of patients with fungal discospondylitis or more systemic fungal infections.

Further Diagnostic Testing

At this level of the investigation the cause of the fever may not yet be apparent. In this situation a systematic approach to further investigation is warranted. This may vary with the individual patient, and your clinical suspicions.

Thoracic Imaging

A physical examination is an insensitive method for thorough examination of the thoracic cavity. Obviously if abnormalities are detected on examination that suggest cardiac or pulmonary disease (cardiac murmurs, pulmonary crackles or wheezes, dull lung sounds or percussion), radiographs are indicated. However even in the absence of obvious intrathoracic disease radiographs are indicated, as there may be occult pulmonary, mediastinal or pleural space disease. It is always important to evaluate all the structures visible on the radiographs, as there may be signs of pathology on the peripheral structures of the radiographs, such as the spine, abdomen, or forelimbs.

Thoracic ultrasonography has limited value for evaluation of normal lungs. It can be helpful though in cases of pleural effusion, cranial mediastinal pathology, or pulmonary masses located adjacent the thoracic wall.

Abdominal Imaging

Every practice would have access to abdominal radiographs. The size and shape of abdominal organs can be assessed, with changes in these parameters suggesting the potential for pathology. Poor abdominal detail in a patient in adequate body condition is suggestive of possible abdominal fluid, peritonitis or neoplasia. Changes in the radiodensity of tissues should be noted – for example gas densities in the liver strongly suggest serious infection. Free gas in the peritoneal space may be a post-operative phenomenon, but may also indicate rupture of the gastrointestinal tract. Poor margination of the prostatic shadow may occur with prostatitis or neoplasia. A tubular fluid/soft tissue density structure in the caudal abdomen of a non spayed female dog should raise the suspicion of a pyometra. As for thoracic radiographs, it is important to evaluate the peripheral structures, such as the spine or pelvis for changes.

Abdominal ultrasonography will be available to a number of practices. This is a useful technique to look for a number of abnormalities in the gastrointestinal tract, viscera or peritoneal space. Ultrasonography can be a sensitive technique to detect peritoneal fluid, masses, enlarged lymph nodes, abscesses or cysts. It typically allows further evaluation of the pancreas and prostate than can be achieved radiographically.

Urine Cultures

If a urinary tract sepsis is considered likely, a urine culture is indicated. In more systemic infections with bacteraemia, considering the high blood flow through the kidneys, the kidneys may become involved. Common organisms in the urinary tract include *E.coli and Proteus spp,* with *Klebsiella spp, Enterococcus spp, Staphylococcus spp* and *Pseudomonas* less common. The method of collection will influence the results of urine cultures. Voided urine samples may be contaminated, and catheterisation can carry bacteria from the external genitalia and skin to the bladder, whereas a cystocentesis sample should be sterile in a normal animal. Urinary tract infection can be eliminated by a negative culture on any sample. Voided samples can have up to 100,000 colony forming units per ml. In up to 20 % of normal female dogs a urine sample collected by catheterisation will yield over 100,000 colony forming units per ml. Therefore cystocentesis samples are the ideal sample for culture.

Blood Cultures

In patients with a fever of unknown origin, where bacteraemia is suspected, or in some diseases where bacteraemia is commonly involved such as discospondylitis, endocarditis or septic polyarthritis, blood cultures are indicated. There are commercially available blood culture bottles that contain appropriate media for both aerobic and anaerobic cultures. Strict aseptic technique should be used when collecting samples. The vein should be clipped and prepared as if for surgery, and sterile surgical gloves used when collecting samples. Previously it was suggested that samples be collected serially over a 24 hour period, but this recommendation has been relaxed, as in human medicine multiple simultaneously drawn samples have been shown to be as effective. The author’s preference is to draw three samples each an hour apart from different sites. When using a syringe cease negative pressure before removing the needle from the vein to minimise contamination from the skin. A new needle should be used to transfer the blood to the culture bottle. The samples should be stored at 37 degrees ideally, or room temperature if waiting to be shipped.

Serology/PCR

Depending on your geographic location, and the clinical signs the patient is displaying, serology for infectious diseases may be indicated. When the author was in the USA, any patient with an appropriate travel history, fever, thrombocytopaenia and hypoalbuminaemia would have Ehrlichia titres performed. Patients with acute renal failure, with or without fever would have leptospiral titres checked. Serology for cryptococcosis, toxplasmosis or neosporosis may be indicated for some patients with neurological, muscular or pulmonary diseases.

Echocardiography

Endocarditis is a relatively uncommon, but serious cause of fever in dogs, with 80-90 % of patients with the disease having a fever. Bacteraemia is the most common aetiology of disease, and the mitral and aortic valves are most commonly affected. Gram negative organisms typically cause a more acute disease, whereas it may be more chronic with gram positive organisms. More commonly implicated organisms include *Staphylococcus, E coli, Streptococcus, Pseudomonas aeroginosa, Corynebacteruim spp, Erysipelothrix rhusiopathiae* and *Bartonella vinsonii.* Clinical signs may be vague and non specific, including fever, lethargy, weakness, inappetance, weight loss and lameness. There may be signs of disease in other organs or tissues related to septic emboli, or deposition of immune complexes. Up to 26% of dogs with endocarditis will not have a heart murmur. Therefore echocardiography should be a consideration if there is a systolic heart murmur noted, or some of the previously mentioned diagnostic tests have not yielded a result. Vegetative valvular lesions may be detected, but the changes may be subtle. Doppler studies can be used to evaluate any valvular insufficiency or outflow tract stenosis.

Fine Needle Aspiration

If during the course of physical examination or imaging studies lesions are detected, fine needle aspiration may be an option. This can be easily achieved with palpable lymph nodes, or other superficial palpable masses. Pulmonary lesions or intra-abdominal lesions may also be aspirated. Intra-abdominal aspirates (e.g. prostate, lymph nodes, liver, renal, or other masses) can be aided with ultrasonographic guidance. There is always the risk of haemorrhage, or spreading the pathology (either infection or neoplasia). Thoracic aspirates may be guided by ultrasound if visible, or deeper pulmonary lesions could be aspirated with fluoroscopic guidance. Intrathoracic aspirates carry similar risks to intra-abdominal aspirates, with the additional risk of pneumothorax. Samples obtained can be submitted for both cytological examination, and culture and sensitivity. It is always preferable to have the cytology results to assist in the interpretation of a culture. In cases of discospondylitis radiographs or fluoroscopy can be used to guide fine needle aspirates of intervertebral discs for cytology and microbiology.

Thoracocentesis/Abdominocentesis

In a patient with a fever and a body cavity effusion it is always important to collect fluid for examination. A fluid analysis can determine if the nature of the fluid is indicative of pyothorax or peritonitis. Pyothorax is typically related to bacterial infection. The source of infection may be transthoracic, parapneumonic, haematogenous, or extension from surrounding tissues (such as the mediastinum, with the oesophagus the most likely source). Common organisms include *E.coli*, anaerobes, and in some cases *Nocardia spp* and *Actinomyces spp.* Peritoneal exudates may be non septic (such as with pancreatitis), or septic, but both can be associated with fever. The differentiation of a septic or non septic exudate is based upon whether micro-organisms are present or not. If septic, the source of infection may be penetrating wounds, the liver or biliary system, the urinary tract, or most likely the gastrointestinal tract.

Pulmonary Cytology

In patients with a cough and pulmonary changes on thoracic radiographs, pulmonary sampling can allow characterisation of the nature of the disease, and if infectious, may allow identification of the causative agent. The options for collection of pulmonary samples include transtracheal aspiration, bronchial wash, bronchoalveolar lavage, or pulmonary fine needle aspiration. Transtracheal aspirates are useful for upper respiratory tract sampling, and avoid oropharyngeal contamination when considering microbiology. Bronchial wash and bronchoalveolar lavage techniques are more useful for bronchial or diffuse alveolar diseases. Mass lesions or some interstitial diseases may be better sampled with aspirates.

Radiography of Other Sites

If there is any sign of back pain on examination, spinal radiographs are indicated. Discospondylitis may affect any level of the vertebral column. It is most commonly bacterial in nature, with *Staphylococcus spp* most common, but other isolates may include *E.coli, or Brucella.* Fungal discospondylitis may occur with *Aspergillus tereus*, most commonly affecting German Shepherds. The most common source of infection is haematogenous, but migrating foreign bodies, likely inhaled rather than ingested, are also a possible source, and they can migrate along the diaphragm to the lumbar spine. As discospondylitis may affect multiple levels of the spine, the whole spine should be radiographed. Typically the positioning and the quality of the radiographs will be improved by general anaesthesia. In cases where polyarthritis is suspected, radiographs of joints to check for erosive changes are indicated.

Advanced Imaging

In some situations standard imaging techniques such as radiography or ultrasonography will provide insufficient information for diagnosis. In some situations advanced imaging such as computerised tomography (CT) scans or magnetic resonance imaging (MRI) will provide improved resolution for diagnosis. CT scanning has significantly improved sensitivity for thoracic or bony lesions than radiographs. MRI scans are useful in many situations including intracranial, spinal or intra-abdominal diseases.

Arthrocentesis

Inflammatory arthritis may be infectious or immune mediated in nature. Infectious arthritis may affect one or more joints. It may affect dogs of any age, but larger breed dogs and males are more commonly affected. The most commonly implicated organisms include beta haemolytic *Streptococci spp, Staphylococcus spp, E.coli, Pasteurella spp, Erysipelothrix spp,* and less commonly *Corynebacterium, Salmonella,* and *Pseudomonas spp,* along with anaerobic organisms. *Mycoplasma* can also cause polyarthritis, as can bacterial l-forms. Other infectious agents, many exotic, that have been associated with polyarthritis include *Borrelia burgdorferi,* rickettsial organisms, and protozoal agents including *Leishmania*, *Hepatozoon canis,* and *Babesia spp.* Immune mediated diseases typically are a polyarthritis, and are the consequence of a type III hypersensitivity reaction. The immune mediated polyarthritides are classified as erosive or non erosive in nature. The erosive forms include rheumatoid arthritis, and an erosive polyarthropathy of Greyhounds. Non erosive forms of the disease may be associated with systemic lupus erythematosus. There are other forms of non erosive disease associated with polymyositis or meningitis that do not meet the diagnostic criteria for SLE. Idiopathic forms of the disease may be associated with no apparent underlying disease, or may be secondary to infections in other tissues, gastrointestinal disease, or distant neoplasia. Disease has been reported after vaccination, or exposure to drugs such as trimethoprim sulphonamide combinations (especially in Dobermans), macrolides, cephalosporins and penicillins. Polyarthritis can also be a feature of Sjogren syndrome, where there may be concurrent keratoconjunctivitis sicca and xerostomia.

In all cases arthrocentesis will be central in the diagnostic evaluation. In fever of unknown origin patients it should be considered a potential part of the diagnostic work-up even in the absence of joint swelling. Ideally fluid from more than one joint should be evaluated. A fluid analysis (if adequate fluid is obtained), or cytology should be performed. In septic arthritis organisms will not always be seen cytologically, and the cytology may be similar with infected or inflammatory joints. There is typically an increase in the cell count, and an increased proportion of neutrophils in the fluid. Therefore a culture is always indicated. The yield from joint fluid can be increased by placing the joint fluid in a blood culture bottle. Concurrent blood cultures are also an alternative.

Cerebrospinal Fluid Collection and Analysis

In patients with cervical or spinal pain and fever, meningitis and discospondylitis are possible causes. If the radiographs do not reveal any suggestion of discospondylitis, a CSF tap should be considered to evaluate for meningitis. Bacterial meningitis is rare, but a steroid responsive suppurative meningitis, recognised more frequently in young, large breed dogs, is more common. The CSF cell counts will be increased, typically with increased proportions of neutrophils. Fungal meningitis with cryptococcosis or protozoal induced disease is also recognised. CSF analysis may reveal cryptococcal organisms, but it is rare to detect protozoa. Bacterial infections may result in a reduced CSF glucose concentration. In addition to a fluid analysis, bacterial culture and serology can be performed on the fluid samples.

Biopsy and Histopathology

The uses of fine needle aspirates and cytology were discussed earlier. In some situations the cytology may be inconclusive, and biopsy of the affected tissue may be indicated. There are some situations where cytology is of limited value. An example would be patients with areas of diffuse cutaneous or subcutaneous swelling. This could be the result of infection, such as with cellulitis, or more diffuse disease such as vasculitis or neoplasms such lymphangiosarcoma or angiosarcoma. If collecting biopsies in this situation a skin biopsy punch is suitable, but it is necessary to make the biopsy deep enough to include both the skin and subcutaneous tissue. In cases with renal proteinuria, renal biopsy can confirm glomerulonephritis, and may help with the prognosis.

Testing for Immune Mediated Disease

In the situation of immune mediated haemolytic anaemia, there are some tests used to try and confirm the disease. A simple patient side test is to check for autoagglutination. Using a glass slide, one to two drops of blood can be placed on the slide, with twice the volume of saline. If rouleaux formation has occurred, the red cells can be distributed through the saline. If autoagglutination is occurring, the clumps cannot be broken up in the saline. Autoagglutination may also be apparent in the EDTA tube – when the blood is mixed in the tube, you can see clumps of cells on the side of the tube. If autoagglutination is detected, it is diagnostic for IMHA. The Coombs test detects immunoglobulin directed against red blood cells, and many people rely on the test to make a diagnosis of IMHA. The test has its limitations however. False positive results can be obtained in patients with other immunologic or non-immunologic diseases, and other causes of anaemia. There may be false negative results, and with some methodologies more antibody is required for a positive test result than is necessary to cause disease. In cases where autoagglutination has been detected, or significant spherocytosis is found in conjunction with anaemia, there is probably no need to perform a Coomb’s tests. However in cases where there is a suspicion of IMHA, it can be used as supportive evidence.

There are other tests that can be potentially used to try and help support a diagnosis of diseases such as systemic lupus erythematosus. The lupus erythematosus cell preparation detects neutrophils that contain phagocytised nuclear material (LE cell). The test is difficult to perform, and is relatively insensitive. Antinuclear antibodies are a mixed population of antibodies directed against various nuclear antigens. There are various assays to detect these antibodies, using frozen sections of rat liver or cultured cell lines. The results are typically expressed as titres. Low ANA titres may be present in normal animals, and may also be present in patients with infectious, inflammatory or neoplastic diseases. The test has limits with both sensitivity and specificity, and there is significant variation in testing techniques, so it is of limited value practically, and alone cannot confirm or rule out a diagnosis of SLE.

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FEVER CASE STUDIES

CASE “TAJ”

Signalment: Six year old castrated male Weimeraner

Presenting Complaint: Haematuria

History: Taj was presented to his primary veterinarian two weeks previously because of haematuria, inappetance and lethargy. A physical examination revealed mild abdominal discomfort and bilateral conjunctivitis. Amoxycillin/clavulanate 500 mg q 12 hours was prescribed. He responded well to therapy, but then became unwell again, with inappetance and lethargy. He was represented, and his temperature was 40 degrees. There was some apparent joint swelling. Blood was collected and submitted for a blood count and biochemical profile (results attached). Intravenous fluids were commenced, with amoxicillin/clavulanate (500 mg per os q 12 hours) and enrofloxacin (200 mg per os q 24 hours). He appeared to become brighter with this therapy. He was discharged, but then developed melaena, and he became painful when walking. He was then referred for further evaluation.

Physical Examination:

Temperature: 39.4

HR = PR = 164

RR = 28

MM = Pink

CRT = 1 sec

Taj was quiet but responsive upon examination. His BCS was 2/5, and there was generalised muscle atrophy. There was mild dental tartar on oral cavity examination. He had bilateral mucopurulent ocular discharge, and hyperaemic conjunctiva. There was mild enlargement of the right prescapular and popliteal lymph nodes. Thoracic auscultation revealed a Grade I/VI systolic heart murmur at the left base. Abdominal palpation revealed a tense abdomen, but no obvious abnormalities. The carpi and hocks were swollen, with swelling of the limbs distal to the joints. The prostate was palpable, and was soft, mobile and non painful, but was slightly larger than expected for a castrated dog.

CBC

CBC		
RBC	**3.9**	(4.9-8.2)
Haemoglobin	**92**	(100-206)
Haematocrit	**0.28**	(0.35-0.58)
MCV	72	(64-76)
MCH	24	(21-26)
MCHC	329	(310-360)
Platelets	Clumped and adequate	
Platelet Count	220	(200-500)
Retic %	0.5%	
Retic Abs	20	(<81)
WCC	**93.9**	(4.5-17.0)
Neutrophil %	96 %	
Neutrophil Abs	**90.1**	(3.5-12.0)
Lymphocyte %	0 %	
Lymphs Abs	**0**	(0.9-3.5)
Monocyte %	3%	
Monocyte Abs	**2.8**	(0.0-1.1)
Eosinophil %	1%	
Eosinophil Abs	0.9	(<1.5)
Basophil %	0%	
Basophil Abs	<0.1	
Plasma protein69	(62-85)	
Plasma appearance:	Normal	
Blood smear examination:	Moderate Toxic-Change. Red cell morphology normal.	

Biochemistry

Sodium	153 mmol/L	(144-154)
Potassium	3.8 mmol/L	(3.8-5.8)
Chloride	111 mmol/L	(93-121)
Bicarbonate	20.3 mmol/L	(14-28)
Anion Gap	25.3 mmol/L	
Urea	2.9 mmol/L	(1.7-6.5)
Creatinine	78 mmol/L	(0.04-0.14)
Glucose	**0 mmol/L**	(2.8-6.7)
Bilirubin	4 umol/L	(<10)
AST	56 U/L	(1-80)
ALT	27 U/L	(1-70)

GGT	18 U/L	(<5)
Alk Phos	116 U/L	(1-120)
Protein	70 g/L	(53-75)
Albumin	25 g/L	(22-36)
Globulin	**45 g/L**	(31-39)
Alb:Glob	0.56	
Calcium	2.48 mmol/L	(2.00-2.80)
Phosphate	1.50 mmol/L	(1.30-2.00)
Creatine Kinase	81 U/L	(<180)
Amylase	1755 U/L	(<2400)
Lipase	**161 U/L**	(1-70)
Cholesterol	4.1 mmol/L	(3.6-8.8)
Serum appearance.	Normal	
Lithium heparin sample.	Glucose result from lithium heparin sample.	

Urine Report

Leucocytes	120 hpf
RBC	>200/hpf
Epithelial cells	5/hpf
Casts	None seen
Crystals	None seen
Debris	Amorphous debris 1+

Urinalysis

SG	1.030
pH	7.0
Protein	3+
Glucose	Negative
Ketones	Negative
Blood	3+
Bilirubin	Negative

CASE “SCULLY”

Signalment: Four Year Old Female Spayed Cattle Dog

Presenting Complaint: Off colour

History: Scully had been lethargic for approximately five days, but would still go for walks, and was eating normally. She then began to gag and cough, and appeared unwell. Examination at the primary veterinary clinic was unremarkable, and Cephalexin, 600 mg every 12 hours was prescribed. Four days later she became more lethargic, inappetant, and vomited food once. She was presented to an emergency clinic, where her temperature was elevated (41.2). Enrofloxacin was added to her drug regime. Upon representation to the primary veterinarian blood was collected and submitted for a blood count and biochemical profile. This detected a neutropaenia (200/uL) and thrombocytopaenia (49,000/uL), and an elevation of ALT (334 u/L) and creatinine (128 umol/L). She then appeared stiff and was walking slowly, so Firocoxib was administered. “Scully” was referred for further evaluation.

Physical Examination:

Temp = 38.1

HR = PR = 80

RR = Panting

MM = Pink

CRT = 1.5 sec

Scully was nervous upon examination. Thoracic auscultation was unremarkable, as was abdominal palpation.

Complete Blood Count

WBC	**1.99**	(5.5-16.9)
Lymphocytes	1.07 x10^9/L	(0.5-4.9)
Monocytes	0.76 x10^9/L	(0.3-2.0)
Neutrophils	**0.15 x10^9/L**	(2.0-12.0)
Eosinophils	**0.03 x10^9/L**	(0.1-1.49)
Basophils	--.-- x10^9/L	(0.0-0.1)
%Lymphocytes	53.4%	
%Monocytes	37.9%	
%Neutrophils	7.3%	
%Eosinophils	1.4%	
%Basophils	0.0%	
Hct	42.5%	(37-55)
RBC	5.7 x 10^12/L	(5.5-8.5)
Hb	14.5 g/dl	(12.0-18.0)
Retic	28.2 X 10^3/uL	
%Retic	0.5 %	
MCV	74.9 fL	(60.0-77.0)
RDW	15.0%	(14.7-17.9)
MCHC	34.1 g/dL	(30.0-37.5)
MCH	25.58 pg	(18.5-30.0)
Plt	**106 x 10^3/uL**	(175-500)
MPV	14.50 fL	
PCT	0.2%	
PDW	26.5%	

CASE “JASPER”

Signalment: Eleven month old male Labrador

Presenting Complaint: Pain

History: One week prior to presentation Jasper had been reluctant to eat, and became lethargic. The following day he presented to his primary veterinarian. Examination revealed a fever (41.0), abdominal discomfort, pain with hip manipulation, elbow pain, and pain on palpation of his spine. Antimicrobials (amoxicillin/clavulanate and metronidazole) and anti-inflammatories (carprofen) were prescribed. There was minimal response, so blood was collected for a blood count and biochemical profile. The blood count revealed a neutrophilia (19,400/uL), lymphocytosis (4,200/uL) and monocytosis (4,500/uL). The only abnormality noted on the biochemical profile was a mild elevation in amylase (1,632 u/L). Radiographs of the elbows, pelvis/hips and thorax were unremarkable. His clinical signs persisted, so he was referred for further evaluation.

Physical Examination:

Temp = 40.7

HR = PR = 100

RR = panting

MM = Pink

CRT = 1 sec

Jasper was quiet but responsive. He had a stiff gait, but no joint swelling was noted. His abdomen was tense upon palpation. There was cervical rigidity, with pain upon palpation of his neck and thoracolumbar spine.

CASE "TOBY"

Signalment: Fourteen month old castrated male Golden Retriever

Presenting Complaint: Unwell

History: Toby was castrated seven weeks prior to presentation, and the procedure and his recovery was uneventful. Three weeks later he was found in the driveway, unable to rise. He was presented immediately to the primary veterinarian. Toby had a very tense abdomen, and a mild fever (39.3). Abdominal radiographs showed foreign material in his stomach. Blood was collected for a blood count and biochemical profile. There was a mild non regenerative anaemia, and a mildly elevated blood urea. He was treated with a non steroidal anti-inflammatory, but the clinical signs persisted. Three days later repeat abdominal radiographs showed persistence of the foreign material in the stomach. An exploratory laparotomy was performed, and sharp plastic was removed from his stomach. Despite the surgery and ongoing antimicrobials (cephalexin and dicloxacillin) his pain persisted. A repeat blood count revealed a persistent non regenerative anaemia (Haematocrit 0.32 L/L). Prednisolone (20 mg every 12 hours) was added to his drug regime. He appeared to respond over a few days to this therapy, but ten days later he became lethargic. Re-examination revealed some joint pain. Cyclophosphamide 50 mg was administered, and he was referred for further evaluation.

Physical Examination:

Temp = 39.4

HR = PR = 108

RR = panting

MM = Pink

CRT = 1 sec

Toby was quiet upon examination, and was in lean body condition (BCS 2/5). The skin on his ventral abdomen was thin, with prominent vessels. Thoracic auscultation revealed a Grade I-II/VI left sided systolic heart murmur at the base. His abdomen was tense, and was therefore difficult to palpate. His carpi were not painful, but he resented manipulation of his stifles, and there appeared to be some effusion. He had hip pain, and some possible back pain.

CBC

Hb	124 g/L	(115-180)
RCC	**4.8 x 10^12/L**	(5.0-8.0)
Hct	**0.35**	(0.37-0.55)
MCV	73 fl	(63-74)
MCH	26 pg	(20-25)
MCHC	354 g/L	(310-360)
Platelets	297	(200-900)
WBC	26.4 x10^9/L	(6.0-14.0)
Neuts	**24.0 x10^9/L**	(4.1-9.4)
Lymph	1.1 x10^9/L	(0.9-3.6)
Mono	**1.3 x10^9/L**	(0.2-1.0)
Eos	0.0 x10^9/L	(0.1-1.2)
Baso	0.0 x10^9/L	(<0.1)
Retics	0.4 %	
Retics abs	19	

Red cells	Anisocytosis+
White cells	Leucocytosis++, Neutrophilia++, Monocytosis+
Platelets	Normal

Biochemistry with Electrolytes

Na	146 mmol/l	(144-160)
K	4.3 mmol/l	(3.8-5.8)
Cl	107 mmol/l	(93-122)
Bicarb	**28.1 mmol/l**	(14-28)
Anion Gap	16 mmol/l	(15-25)
Urea	**10.1 mmol/l**	(1.7-6.5)
Creatinine	56 umol/l	(40-140)
Glucose	6.2 mmol/l	(3.5-6.7)
Bilirubin	3.5 umol/l	(3.0-22.0)
AST	23 U/l	(1-80)
ALT	**88 U/l**	(3-70)
AlkPhos	**516 U/l**	20-155)
T Protein	63 g/l	(53-75)
Albumin	31 g/l	(23-39)
Globulin	31 g/l	(31-39)
Calcium	2.40 mmol/l	(2.0-2.8)
Phosphate	1.40 mmol/l	(1.3-2.0)
CreatKinase	38 U/l	(0-180)
Amylase	299 U/l	(1-2400)
Lipase	938 U/l	(250-1750)
Cholesterol	5.40 mmol/l	(3.6-8.8)

Urinalysis

Specimen: Catheter

Chemistry
pH: 8.0
Protein: Trace
Glucose: Nil
Blood: Nil
Ketones: Nil
Crystals: No crystals seen
Specific Gravity: 1.014

Microscopy

Leucocytes	3 x10^6/L	(<10)
Erythrocytes	0 x10^6/L	(<10)
Epithelial cells	0 x10^6/L	(<10)
Bacteria	Nil	

CASE “JERRY – LEE”

Signalment: Two year old castrated male German Shepherd

Presenting Complaint: Fever

History: Jerry Lee had a three day history of lethargy, heavy breathing, hypersalivation, nasal discharge, and bloodshot eyes. He was typically a very active dog, but had been reluctant to undertake any activity. He was also inappetant. He was presented to the primary veterinarian, who detected a fever (39.6), and possible cervical pain. A blood count (attached) revealed a neutrophilia and monocytosis. There was a mild hypoglycaemia on a biochemical profile. Therapy was commenced with intravenous fluids and cephazolin. Radiographs were taken of the cervical spine and thorax, and both were unremarkable. Jerry Lee was referred for further evaluation.

Physical Examination:

Temp = 39.4

HR = PR = 100

RR = panting

MM = Pink

CRT = 1 sec

Jerry Lee was quiet upon examination. He was able to walk, but preferred to lay down. There was no apparent thoracolumbar pain. There was no pain on cervical palpation, but he resented strong dorsiflexion of his neck. Thoracic auscultation revealed a Grade I/VI systolic heart murmur at the left base. Abdominal palpation was unremarkable. There was some swelling of his carpi and hocks, and he was uncomfortable on manipulation of these joints and the elbows. The palpable lymph nodes were all prominent upon palpation.

CBC

RBC	5.6	(4.9-8.2)
Haemoglobin	138	(100-206)
Haematocrit	0.41	(0.35-0.58)
MCV	73	(64-76)
MCH	25	(21-26)
MCHC	337	(310-360)
Platelets	Clumped	
Platelet Count	43	(200-500)
WCC	22.3	(4.5-17.0)
Neutrophil %	81 %	
Neutrophil Abs	**18.1**	(3.5-12.0)
Lymphocyte %	2 %	
Lymphs Abs	**0.4**	(0.9-3.5)
Monocyte %	16%	
Monocyte Abs	**3.6**	(0.0-1.1)
Eosinophil %	0%	
Eosinophil Abs	0.1	(<1.5)
Basophil %	1%	
Basophil Abs	0.2	
Plasma protein	79	(62-85)

Plasma appearance: Normal

Blood smear examination: Slight toxic Change. Red cell morphology normal.

ALB = 33 G/L
ALKP = 131 U/L
ALT = 41 U/L
AMYL= 1238 U/L
UREA = 3.4 mmol/l
Ca = 2.62 mmol/l
CHOL = 7.38 mmol/l
CREA = 75 umol/l
GLU = 3.56 mmol/l
PHOS = 1.46 mmol/l
TBIL < 2 umol/l
TP = 68 g/l
GLOB = 35 g/l
PCV 42%

Urinalysis
Specific Gravity 1.010
pH = 5.5
protein = negative
glucose = negative
ketones = negative
blood = trace
Occ white blood cells, no bacteria

CASE “BUSTER”

Signalment: Nine month old male Great Dane

Presenting Complaint: Skin Disease

History: Archie was obtained at nine months of age. He occasionally had bruises on his side, but this was attributed to self trauma as they would spontaneously resolve. Six weeks prior to presentation he had swelling of his left forefoot. Examination revealed a mild fever, but was otherwise unremarkable. He was treated with cephalexin, and the swelling settles somewhat, but did not resolve. In the week prior to presentation all four feet became swollen. Amoxycillin/Clavulanate was administered, but after two doses he developed generalised cutaneous erythema. Prednisolone 40 mg was administered for two doses, but there was no response. Norfloaxcin was then administered. He was represented to the primary veterinarian, who detected distal limb and ear pinna swelling. There had been some facial pruritis. He was not lame. He was referred for further evaluation.

Physical Examination:

Temp = 39.4

HR = PR = 120

RR = panting

MM = Pink

CRT = unable to determine

Buster was somewhat apprehensive, so a full examination was not possible. The prescapular and popliteal lymph nodes were enlarged. Thoracic auscultation was unremarkable. Abdominal palpation revealed hepatomegaly, splenomegaly, and some ‘nodular’ areas, that were considered to likely be lymph nodes. The ear pinnae were erythematous and thickened. There was swelling of both forefeet, worse on the left. There was marked cutaneous erythema ventrally, around the elbows, and the antebrachia.

HAEMATOLOGY

				Reference Range
RBC		5.9	x1012/L	(4.9-8.2)
Haemoglobin		128	g/L	(100-206)
Haematocrit		0.40	L/L	(0.35-0.58)
MCV		68	fL	(64-76)
MCH		22	pg	(21-26)
MCHC		320	g/L	(310-360)
Platelets		Clumped and decreased		
Platelet Count		**8**	L x109/L	(200-500)
WCC		**1.6**	L x109/L	(4.5-17.0)
Neutrophil	15%	**0.2**	L x109/L	(3.5-12.0)
Lymphocyte	82%	1.3	x109/L	(0.9-3.5)
Monocyte	2%	**< 0.1**	x109/L	(< 1.2)
Eosinophil	1%	< 0.1	x109/L	(< 1.5)
Basophil	0%	< 0.1	x109/L	
Plasma Protein		62	g/L	(62-85)
Plasma Appearance		Normal		

Red cell and white cell morphology normal.

Platelets are clumped but appear significantly decreased on review of the blood film. There is a marked neutropenia. There are some reactive lymphocytes.

Biochemistry with Electrolytes - Canine

Na	145 mmol/l	(144-160)
K	**3.5 mmol/l**	(3.8-5.8)
Cl	114 mmol/l	(93-122)
Bicarb	23.1 mmol/l	(14-28)
Anion Gap	12 mmol/l	(15-25)
Urea	2.9 mmol/l	(1.7-6.5)
Creatinine	73 umol/l	(40-140)
Glucose	5.0 mmol/l	(3.5-6.7)
Bilirubin	3.6 umol/l	(3.0-22.0)
AST	24 U/l	(1-80)
ALT	41 U/l	(3-70)
AlkPhos	118 U/l (20-155)	
T Protein	53 g/l	(53-75)
Albumin	24 g/l	(23-39)
Globulin	**29 g/l**	(31-39)
Calcium	2.49 mmol/l	(2.0-2.8)
Phosphate	1.93 mmol/l	(1.3-2.0)
Amylase	756 U/l (1-2400)	
Lipase	557 U/l (250-1750)	
Cholesterol	2.79 mmol/l	(3.6-8.8)

Urinalysis

Urine Volume (ml)	10.0
Urine Colour	Yellow
Urine Transparency	Transparent
Urine SG	1.034
Urine Glucose	Negative
Urine Bilirubin	+ (small)
Urine Ketones	Negative
Urine Haemoglobin	Trace
Urine pH	8.5
Urine Protein	+ (0.3 g/L)
Urine Urobilinogen	Normal (less than 20umol/L)
Urine RBC/HPF	28.0
Urine WCC/HPF	< 1.0
Urine Casts, Number	Negative
Urine Bacteria	Negative
Urine Fat	Negative
Urine Epithelial Cells, Type	Transitional
Urine Epithelial Cells, Number	Few
Urine Epithelial Cells, Number	Negative
Urine Sperm	Occasional
Urine Crystals, Type	Struvite
Urine Crystals, Number	Moderate

Hypersthenuria reflects adequate urinary concentration ability. Mild haematuria and proteinuria without actives sediment.

NOTES:

Chapter 3

Anaemia

Dr Graham Swinney

Anaemia is a clinical sign of disease that is common in canine medicine. It may be the result of a specific erythrocyte disorder, or may be the result of systemic disease. By definition anaemia is a decrease in the red blood cell (RBC) mass as shown by a reduction in the RBC count, haematocrit or haemoglobin concentration. The clinical signs of anaemia may be the consequence of reduced oxygen delivery to tissue, a reduced blood volume, but may also reflect an underlying disease process. When classifying anaemias, it is typically done by pathophysiologic mechanisms, red cell indicies, and an assessment of the bone marrow response. The main classification is whether the anaemia is regenerative or not.

The red cell count is the result of a balance between red cell production and red cell removal or loss. If production reduces, or removal increased, anaemia will likely be the result. Typically if anaemia develops the body will try to increase erythropoiesis. The kidneys will produce erythropoietin which acts on the burst forming units-erythroid in the bone marrow. Erythropoietin (EPO) stimulation can increase red blood cell production in the marrow ten to fifty fold. The maturation from the earliest committed erythroid precursor to a mature erythrocyte is around 7 days.

Reticulocytes are the typical marker used to indicate regeneration in dogs. They are immature red cells that can continue to synthesise haemoglobin as long as messenger RNA is present. Reticulocytes typically reside in the marrow for two days before release. In situations where erythropoiesis is accelerated, they will be released earlier, and will spend longer maturing in circulation. The presence of the RNA allows them to be identified by supravital stains such as new methylene blue.

THE APPROACH TO AN ANAEMIC PATIENT

Because of the many different causes of anaemia, and the variable severity of disease, there will not always be a typical presentation of an anaemic patient. However the general approach can be discussed. Specific features relative to particular diseases will be discussed in the more detail later.

Signalment

The signalment of a patient may have relevance in some anaemic patients. With regenerative anaemias there are certain breeds of dog predisposed to primary immune mediated haemolytic anaemia (IMHA). Certain breeds may be at risk for inherited disorders resulting in anaemia. Older patients may be more likely to have an underlying disease resulting in anaemia. Female dogs are more likely to be affected by immune mediated disease.

History

Patients with anaemia may present with non specific clinical signs of lethargy, episodic weakness, reduced exercise tolerance or collapse. Owners may also notice secondary signs such as tachypnoea. Changes in urine colour (yellow, pink), or even changes in mucous membrane (pale, icteric), or skin colour (icterus) may be noted. The history may provide information on a precise aetiology for the anaemia, for example a known history of trauma, or an exposure to an anticoagulant rodenticide. The duration of the clinical signs may give some clue to the nature of disease (acute vs chronic). The collection of a drug and vaccination history may prove important. The history may also provide indication of a possible underlying disease process (e.g. altered water consumption, weight loss, diarrhoea, melaena, haematochezia).

Physical Examination

The physical examination is very important to help recognise a potential for anaemia (pale membranes), to help determine the clinical effect it is having (heart rate, respiratory rate, activity and demeanour), and potentially detect an underlying cause. A full physical is essential, but particular attention should be paid to the membranes, skin (haemorrhages), thoracic auscultation (murmurs, crackles, indications of pleural fluid), abdominal palpation (fluid, liver, spleen), lymph node palpation, and a rectal examination (faecal colour). Another important step once anaemia has been defined is to relate the patient’s clinical signs to their PCV. A patient with a low PCV that appears to be managing well clinically likely has a degree of chronicity to the anaemia, whereas a patient with an acute onset of anaemia may have more severe clinical signs with a moderate drop in PCV.

Laboratory Evaluation

Haematology

At the most basic level, a packed cell volume should be checked, as it will define if anaemia is present or not. It is always very important to check a total protein concurrently. A fall in the total protein is suggestive of blood loss. This can even be important if monitoring a patient where the cause of anaemia is known – a patient being immunosuppressed for a haemolytic cause of anaemia that has a plateau or fall in PCV, that also has a falling total protein may have developed gastrointestinal ulceration, with ensuing blood loss.

With the advent of many in-house haematologic instruments, many practitioners now perform many of the routine blood count analyses in-house. Obviously there are numerous advantages, including financial, and not the least rapid turn around times for results. There are some important factors to consider however when using such instruments. These instruments require routine maintenance, quality control and calibration. For example it is recommended that two different control samples should be run during an eight-hour period of patient testing, or any time reagents are changed. The instrument should be calibrated after a major service, if indicated be quality control data, if changing reagent type, or at least every six months. If these are not performed, the results of the instruments may be called into question.

The automated in house analysers do not provide a review of a blood smear. In some cases this will not be a problem. In other cases this will be a huge issue, as it will mean you are missing vital information. A smear review in an anaemic patient that reveals many acanthocytes could increase suspicion of a disease process such as haemangiosarcoma. Anisocytosis and polychromasia are generally suggestive of a regenerative response, whereas spherocytes suggest haemolytic disease.

Therefore it is strongly recommended to include a review of a blood smear as part of any in-house CBC. Obviously if any unusual cell morphology is detected, it is worthwhile to send a blood sample to a laboratory with a specialist clinical pathologist for review.

In anaemic patients, review of the red cell indicies is a very important. Ideally a reticulocyte count will also be evaluated, but in the absence of this, the indicies may give some clues as to the nature of the anaemia. In a typical regenerative anaemia, there will be a macrocytosis (elevated MCV) and hypochromasia (reduced MCHC). It is important to remember that the absence of macrocytosis does not rule out the possibility of a regenerative response. The presence of regeneration, as evidenced by increased reticulocytes, in the face of a normal MCV, is suggestive of the presence of a disease often associated with microcytosis. Most commonly this will be some cause of chronic blood loss, or a bleeding disorder. A normocytic-normochromic anaemia is more typically non regenerative in nature.

The reticulocyte count will be an important part of the evaluation of an anaemic patient. The reticulocyte count may be expressed as a percentage, or as an absolute count. The absolute count is the preferable way to assess the degree of regeneration. The degree of regeneration should be related to the severity and duration of anaemia. Remember that even with diseases typically associated with a regenerative response, the regenerative response may be absent very early in the course of the disease, so the anaemia may be considered ‘pre-regenerative’. A more severe anaemia will result in increased EPO levels compared to a mild anaemia. In mild anaemias, the regenerative response may be mild. There have been methods described to correct the reticulocyte count for the degree of the anaemia, but they are more complicated, and involve correction of the absolute count based on the current PCV and maturation times for reticulocytes in peripheral blood at the current PCV (longer at lower PCV’s).

The presence of nucleated red blood cells on a smear does not always mean regeneration. They may be part of a more intense regenerative response in some situations, but they can also occur with bone marrow pathology, splenic disease, lead intoxication, and may also be breed associated (Miniature Schnauzers).

There are some more common causes of microcytosis in small animal practice. These include iron deficiency anaemia, most commonly associated with chronic blood loss. In such cases the gastrointestinal tract is the most common culprit, even in the absence of obvious clinical signs such as melaena. Other possible sources of chronic blood loss are the respiratory and urinary tracts. Portosystemic shunts in both cats and dogs are often associated with microcytosis, probably related to iron redistribution rather than iron deficiency. More specifically, there are some breed related cases of microcytosis, notably the Japanese Akita, and the Shiba Inu.

Biochemical Profile/Urinalysis

A biochemical profile and urinalysis may reveal signs of an underlying disease process that could be causing the anaemia (for example azotaemia and isosthenuria). These tests may also reveal signs of organ damage as a result of anaemia (elevated liver enzymes, acidosis). The presence of bilirubinuria and hyperbilirubinaemia may suggest haemolysis.

Other Testing

Depending upon the nature of the disease, other testing may be indicated. Thoracic (radiographs) and abdominal (radiographs and ultrasound) imaging may be indicated to look for a source of haemorrhage, or underlying disease. Fine needle aspiration and cytology of abnormal organs or tissues (e.g. enlarged lymph nodes, liver, spleen, masses) may be necessary, and in some situations biopsy of affected organs. Coagulation testing may be necessary, as could evaluation of serum iron parameters. Geographically serologic testing may be indicated. Serum levels of Vitamin B12 are occasionally required. Endocrine testing (serum total or free T4, TSH levels, or adrenal function testing) may be indicated based upon clinical examination or other laboratory tests.

Bone Marrow Evaluation

Bone marrow aspirates may be indicated in cases of non-regenerative or poorly regenerative anaemia. There are a number of sites that can be employed, including the proximal humerus, wing of the ilium, or the femur.

Materials required include bone marrow aspirate needles (that have a stylet), or a Jam Shidi bone biopsy needle, some sterile anticoagulant, and a watch glass or dish in which to place the sample. The needle should be flushed with EDTA. After insertion of the needle (with the stylet in place), it should be firmly seated in the bone. Once the stylet is removed, a syringe is attached, and aspiration performed. Once marrow enters the syringe, stop aspirating, and squirt the marrow into the watch glass that also contains EDTA, and mix. Smears can then be made of the marrow, and marrow material left over can be placed into an EDTA blood tube for submission. If using a Jam Shidi needle, and a poor sample is obtained upon aspiration, the needle can be advanced with the stylet out, and a bone marrow core sample can be collected. This can be rolled on a slide for cytologic examination, and can be placed in formalin for histopathologic examination.

CAUSES OF ANAEMIA

Regenerative Anaemias

Anaemia that is regenerative will be the consequence of either haemorrhage or haemolysis.

Haemorrhage Associated Anaemia

Blood loss may be generalised or localised in nature. The clinical signs depend on the volume and rate of blood loss. Acute and chronic blood losses are both regenerative, but the regeneration may be limited initially, and may become poorly regenerative with chronic loss associated with iron deficiency.

Acute Haemorrhage

If acute haemorrhage is severe, the patient will be compromised not just by the loss of red blood cells, but also by the loss of volume, and signs of hypovolaemic shock may occur. The body will preferentially attempt to maintain perfusion to the brain and heart, and then viscera. Perfusion of skin and muscle will reduce, and the spleen will contract. The heart rate will increase to try and maintain perfusion with sympathetic stimulation. The greater the loss the more marked are the clinical signs – losses of <20% of blood volume may be tolerated well, but if losses approach 50% acutely, most patients will die from hypovolaemia without therapy.

Initially with acute blood loss the PCV/TP will remain normal as whole blood has been lost. It is not until fluid moves from the extravascular space, or fluids have been administered, that the PCV/TP will begin to fall. After a single, acute loss of blood it may take 2-3 days to fully correct the volume deficit.

Initially the anaemia will be normocytic-normochromic, but the erythropoietin levels rise within hours, and by 2-3 days the anaemia will become regenerative. A maximal reticulocyte response may take 4-7 days. Platelet counts may fall acutely, but will rapidly normalise, and a reactive thrombocytosis may even develop. The leucogram will be variable, but a stress response may be noted. The total protein will typically fall, with a panhypoproteinaemia noted.

The cause of acute haemorrhage may be related to trauma or surgery. If generalised haemorrhage is noted, haemorrhagic disorders should be pursued. If there has been haemorrhage into a body cavity, the most likely causes are trauma, a bleeding disorder, or neoplasia. In some cases there may be indicators on the blood smear of underlying disease, for example the presence of acanthocytes. Imaging studies may be required to help detect underlying pathology.

Therapy is acutely directed at the restoration of circulating volume, and the control of ongoing haemorrhage if possible. Alternatives for replacement of volume include crystalloid fluids, colloids (natural or synthetic), or whole blood. Typically in the acute situation crystalloid fluids of a replacement type are the initial choice. The volume required will vary from patient to patient, but because these fluids will also redistribute to the extravascular space the volume required is two to four times the volume lost. Shock rates may be required initially (90 mls/kg/hour). It is important to make sure that fluids being administered at this rate are low in potassium. Synthetic colloid solutions can provide smaller volume resuscitation, but care must be taken in patients with haemorrhagic tendencies. Plasma can also be used if necessary. Overseas some haemoglobin solutions (Oxyglobin) will be used as they provide oxygen carrying support, and are effective colloids.

If haemorrhage is very severe, a transfusion may be indicated. There is no set value for PCV below which a patient should be transfused. Patients with very acute loss may show signs of compromise from reduced oxygen carrying capacity at PCV's over 20%. The decision should therefore be made based upon clinical signs. Packed cells could be used if volume has been replaced, or fresh whole blood can help both oxygen carrying capacity and volume restoration. If venous access is not available, red cells can be administered intraosseously. In an acute situation in a patient with no prior transfusion history, a cross match is not imperitive.

Obviously if there is a known underlying disease it must be treated. This may include therapy of a coagulopathy, or removal of a bleeding neoplasm.

Typically patients that have undergone acute blood loss have adequate iron stores, and iron supplementation will not be required. It may take 1-2 weeks for the PCV to return to pre-haemorrhage levels.

Chronic Blood Loss

Chronic blood loss may result in iron deficiency. The majority of the body’s iron is in the RBCs as haemoglobin, but other locations include muscle myoglobin or some enzyme systems. Iron storage sites are in the spleen, liver and bone marrow, where the iron may be soluble (ferritin), or insoluble (haemosiderin). Transferrin is the iron transport protein in the plasma. Iron is absorbed from the GI tract by the small intestine, but the amount absorbed is relatively small.

Chronic blood loss that results in iron deficiency is typically external, with the gastrointestinal tract the most common site, but the urinary tract can also be involved. Iron depletion occurs when iron stores become depleted, but serum iron and haemoglobin concentrations remain normal. Iron deficient erythropoiesis results in low serum iron, but only mild anaemia. Iron deficiency anaemia is characterised by seriously depleted or absent serum iron stores, reduced serum iron concentration, reduced transferring saturation, and low haemoglobin concentration and PCV.

Aetiology of chronic blood loss can be GI diseases (ulceration, inflammation, neoplasia, severe malabsorption), chronic bleeding neoplasms, endo and ectoparasites (e.g. hookworms and fleas), or may be iatrogenic in small, sick, hospitalised patients, or in blood donors. As mentioned before, the chronic loss may also be via the urinary system (often neoplastic).

Because the anaemia typically is slow in onset, the clinical signs may be insidious. Patients may present with lethargy and reduced exercise tolerance. History may reveal suggestions of gastrointestinal disease, or melaena. Some patients may also present with pica. The clinical examination will reveal pale mucous membranes. Patients may be tachypnoeic, have hyperdynamic pulses, and have haemic heart murmurs. Some patients may develop myocardial hypertrophy chronically. Ectoparasites may be apparent in some patients.

Diagnostic evaluation will often involve a complete blood count. The syndrome is characterised by a microcytic, hypochromic anaemia, which is caused by reduced haemoglobin synthesis, delayed cell maturation and extra mitosis. The hypochromic cells have increased central pallor on a blood smear. In severe cases the red cells may appear as a ring only. The iron deficient red cells are stiffer and less deformable, so RBC fragmentation may occur. There may be a marked reticulocytosis in some patients as a consequence of delayed maturation. The leucogram may be normal. Some gastrointestinal diseases may cause an eosinophilia. A thrombocytosis is common and may be reactive in nature.

Biochemical profiles may be normal, but in some cases a panhypoproteinaemia may be noted.

Serum iron concentrations will typically be reduced, but other factors such as haemolysis or recent transfusion may falsely elevate the values. The iron binding capacity reflects the amount of transferring in the blood, and will usually be elevated. Transferrin saturation will be markedly reduced. Ferritin levels may be reduced, but as it is an acute phase reactant protein, levels may also rise with the underlying disease.

A bone marrow evaluation may reveal mild to moderate erythroid hyperplasia. The normoblasts may appear small. Stains for iron will reveal reduced iron stores.

Treatment is directed at the underlying disease and correcting the iron deficiency. Transfusions are only indicated if the patient is significantly compromised by the anaemia. Iron supplementation will be required to help replenish iron stores. Ferrous sulphate can be administered orally at a suggested dose of 10 mg/kg with food, but the dosages are not well established. Treatment will need to be over a prolonged period. If GI absorption is impaired, iron may be administered parenterally in the form of iron dextran.

Haemolytic Anaemias

Erythrocytes have a finite lifespan in circulation. The average lifespan of canine erythrocytes is 100 to 120 days. This lifespan may be reduced by an intrinsic defect in the cell, or something extrinsic that causes premature removal of the cell.

When a red cell is degraded, the iron is released, bound to transferrin, and is transported in the plasma back to the marrow for erythropoiesis. Biliverdin is also formed, and is converted to unconjugated bilirubin. This is released into the plasma where it travels bound to albumin. It is absorbed by hepatocytes, where it is conjugated with glucuronic acid to make conjugated bilirubin (bilirubin diglucuronide). This is excreted into the bile canaliculi. If there is massive haemolysis, the excretion into the canaliculi is overwhelmed and conjugated bilirubin leaks into the plasma.

If there is intravascular haemolysis haemoglobin is released into the blood. Some is metabolised in the liver to bilirubin, but some is excreted via the kidneys.

Immune mediated haemolytic anaemia (IMHA) is generally the consequence of a type II hypersensitivity reaction. IMHA may be classified based upon the site of haemolysis (extravascular vs intravascular), or as primary or secondary. In primary disease the immune mediated response is truly autoimmune, with the target being the red cell membrane. In secondary disease there may be indirect attachment of immunoglobulin to an antigen, which is adsorbed onto the red cell membrane, or possibly attachment of formed immune complexes. This may be secondary to infections (such as *Mycoplasma, Babesia,* leptospirosis, sepsis, dirofilariasis, ancylostoma), drugs (for example sulphonamides, cephalosporins, penicillins), biologic products such as vaccines, inflammatory disease, or neoplastic disease (such as lymphosarcoma). IMHA may be part of some generalised immune mediated diseases, such as Systemic Lupus Erythematosus.

Immune mediated haemolytic anaemia can be further classified into one of five categories, based on the nature of the disease.

Class I – In saline acting agglutinins

Class II – Antibody can fix complement, so get intravascular haemolysis (typically IgM)

Class III – Extravascular haemolysis

Class IV – Cold reacting agglutinating antibody

Class V – Cold reacting intravascular haemolysis

The majority of cases of IMHA fall into the category of extravascular haemolysis. The erythrocytes with antibody attached interact with phagocytic cells. Macrophages have receptors for the Fc receptor on antibody, or complement. After this attachment, there can be phagocytosis of the cell by the monocyte-macrophage system, or removal of part of the erythrocyte membrane. The latter results in the formation of spherocytes (so the cells will appear smaller with the loss of the typical central pallor). The spherocytes are less deformable than normal red cells, so are more likely to be trapped in the spleen and removed from circulation. In the case of intravascular haemolysis, macrophages do not have a Fc receptor for IgM, and so complement is fixed, and intravascular destruction is the result.

Some cases with immunologically mediated anaemia will present with a non-regenerative anaemia. In this setting the immune response is directed against cells in the erythrocyte line in the bone marrow. This condition may be referred to as Pure Red Cell Aplasia.

It is important to remember that not all haemolysis is immunologically mediated. Oxidative injury to red blood cells causing Heinz bodies may result in haemolysis. In dogs onion ingestion is the most common cause. Propylene glycol in semimoist food also has the potential to cause oxidative damage. Due to the increased number of sulphydryl groups in feline red blood cells, they are very sensitive to oxidative injury. Potential causes include methylene blue, propylene glycol, paracetamol, Vitamin K3, copper, or some diseases such as lymphosarcoma, diabetes mellitus, or hyperthyroidism.

In dogs, some inherited metabolic disorders can be associated with haemolysis, including pyruvate kinase deficiency (Basenjis, Beagles, West Highland White Terrier, Cairn Terrier), or phosphofructokinase deficiency (English Springer Spaniels and Cocker Spaniels). Both enzymes are involved in the glycoloytic pathway which is the primary energy source for erythrocytes. In PFK deficiency, the red blood cells are pH sensitive, with haemolysis more likely to occur with alkalaemia, so episodes can be precipitated by exercise and panting. Signs of a problem may include anaemia, icterus, lethargy, anorexia, fever and dark urine (both haemoglobinuria and bilirubinuria). In PK deficiency there may be persistent pallor, but the clinical signs are typically mild. There are often high reticulocyte counts. In time affected animals may develop myelofibrosis and osteosclerosis.

Zinc can be also be associated with haemolysis, but the mechanism is likely immune mediated. In the USA it is typically associated with the ingestion of pennies, as the gastric acid causes release of zinc. There is resultant membrane damage and haemolysis. Treatment involves removal of the foreign body and antioxidants.

Other metabolic causes have also been implicated, such as hypophosphataemia associated with insulin administration to patients with diabetic ketoacidosis, or refeeding syndrome. The hypophosphataemia likely results in intracellular depletion of ATP. Haemolysis can also be mechanical, such as with some microangiopathies, for example haemangiosarcoma.

Infectious causes of haemolysis are much less common in cats than dogs. *Haemobartonella canis* can be transmitted by the brown dog tick. Clinical signs of anaemia will only develop in dogs that have undergone splenectomy or have another serious illness. Overseas Babesiosis can be an important cause of anaemia. The organism is transmitted by ticks, which must feed for a minimum of two days for the organism to be transmitted to the host. After an incubation period of ten to twelve days patients may develop haemolytic anaemia, hypotensive shock, and multiple organ dysfunction.

In the case of primary IMHA, younger to middle aged animals are more commonly affected, with females being more commonly affected. There are some breeds that appear to be over-represented, including Cocker Spaniels, Springer Spaniels, Collies, Poodles, Old English Sheepdogs, and Irish Setters.

In animals with secondary IMHA, there is not a typical signalment, as it will vary with the initiating disease.

In most cases the clinical signs will have an acute onset. There will be the historical signs of the anaemia, including exercise intolerance, weakness, and possibly collapse. On clinical examination signs may include pale mucous membranes, icterus, tachycardia, tachypnoea, splenomegaly, and possibly fever. If the anaemia is severe enough, there may be a heart murmur noted – typically systolic, due to altered blood viscosity.

There are some initial steps that can be taken in house to formulate an initial diagnosis in a patient with IMHA. Obviously there will be anaemia, which can be confirmed by checking a PCV. It is always a good idea to check the total protein as well. In the situation of a regenerative anaemia, the possible aetiologies include haemorrhage or haemolysis. In cases with haemorrhage whole blood will be lost, so the total protein will also fall. In cases of haemolytic disease, the total protein should not fall. Checking the plasma colour is also important. In many cases with extravascular haemolysis the plasma will be icteric. In the situation of intravascular haemolysis there will likely be haemoglobinaemia, with pink plasma. A blood smear examination can also be performed in house. In the situation of a regenerative anaemia there will likely be anisocytosis and polychromasia as part of the regenerative response. The presence of spherocytes is also suggestive of IMHA. A simple patient side test is to check for autoagglutination. Using a glass slide, one to two drops of blood can be placed on the slide, with twice the volume of saline. If rouleaux formation has occurred, the red cells can be distributed through the saline. If autoagglutination is occurring, the clumps cannot be broken up in the saline. Autoagglutination may also be apparent in the EDTA tube – when the blood is mixed in the tube, you can see clumps of cells on the side of the tube. If autoagglutination is detected, it is diagnostic for IMHA.

A complete blood count will be an important part of the evaluation of a patient with IMHA. Obviously there will many of the features just described above. A reticulocyte count should be performed, and should be elevated, unless it is too soon for a regenerative response, or there are also bone marrow effects. The MCV will be increased secondary to the reticulocytosis. The regenerative response will tend to be more intense with haemolytic disease than with haemorrhagic disease. A neutrophilic leucocytosis, perhaps with a left shift may be detected. The neutrophilia may be secondary to the inflammatory nature of the disease process, and also the intense marrow stimulation secondary to the anaemia. The platelet count may be normal, or could be reduced if there is concurrent immune mediated thrombocytopaenia. As mentioned previously, there may be patients that present with a non-regenerative form of the disease.

Other diagnostic evaluations performed may vary from case to case. A biochemical profile will often be performed. There may be elevations in the liver enzymes (ALT, ALP) secondary to hypoxic damage to hepatocytes, and possibly azotaemia, which may be pre-renal from dehydration or renal secondary to hypoxia or damage from hyperbilirubinaemia. If there is severe anaemia there may also be a metabolic acidosis secondary to reduced oxygen delivery to tissues. Hyperbilirubinaemia will commonly be detected. The bilirubin may be mainly conjugated, as the rate limiting step in bilirubin metabolism is release of conjugated bilirubin form the hepatocyte into the sinusoids. If a urinalysis is performed, there will typically be bilirubinuria if the haemolysis is extravascular, and there may be haemoglobinuria if intravascular haemolysis has occurred. If there is secondary renal damage proteinuria may be detected, and bilirubin crystals may also be noted. The urinalysis may also be useful in detecting underlying disease, for example if there is pyuria or bacteriuria present.

The Coombs test detects immunoglobulin directed against red blood cells, and many people rely on the test to make a diagnosis of IMHA. The test has its limitations however. False positive results can be obtained in patients with other immunologic or non-immunologic diseases, and other causes of anaemia. There may be false negative results, and with some methodologies more antibody is required for a positive test result than is necessary to cause disease. In cases where autoagglutination has been detected, or significant spherocytosis is found in conjunction with anaemia, there is probably no need to perform a Coomb's tests. However in cases where there is a suspicion of IMHA, it can be used as supportive evidence.

If a secondary IMHA is suspected, a search for underlying disease is warranted. Such a search may include testing mentioned previously (CBC, biochemical profile and urinalysis). Imaging of the thorax (radiographs) and abdomen (radiographs or ultrasound) may be warranted. Serology to search for infectious diseases will vary geographically (Haemobartonella, Babesia, Rickettsial organisms, Leptospirosis). Splenic and hepatic aspirates may be indicated if abnormalities are seen. In simple IMHA, splenic aspirates may show signs of extramedullary haematopoiesis. Lymph node aspirates may be indicated in some cases, as would echocardiography if a heart murmur failed to resolve with correction of the anaemia. The clinical signs that the patient may be displaying would indicate other testing.

In the situation of a non-regenerative anaemia, a bone marrow aspirate or biopsy would be indicated. Typically a maturation arrest will be detected at a particular stage of erythrocyte maturation. There will be hyperplasia of the stages present. No cells with neoplastic features should be seen. Erythrophagocytosis may be noted.

If the IMHA has been demonstrated to be secondary to another disease, therapy should be directed against the primary condition if at all possible. In the case of a patient with an infectious disease for example, antimicrobials would be used for treatment. However the IMHA itself may need to be treated as well.

Immunosuppression is the basis of therapy for IMHA. The control of destruction of the erythrocytes can be achieved by decreasing the destruction of antibody-coated cells by the mononuclear phagocytic system, or by reducing the production of anti-erythrocyte antibodies.

Glucocorticoids represent the most common therapy for IMHA. The glucocorticoids suppress erythrophagocytosis, rather than significantly reducing antibody production. Glucocorticoids can be administered intravenously or orally. Whilst there are a number of different choices for glucocorticoids, I routinely use prednisone/prednisolone (either orally or injectably). The dose rate needs to be immunosuppressive, which for prednisone is between 2 and 4 mg/kg/day. This dose may be divided, and I usually start at the lower end of the dose range. The length of time to see a response is often 3 – 7 days, but may take up to 14 days. Side effects of glucocorticoids are well known, including PU/PD, polypahagia and gastrointestinal ulcers. Whilst H2 blockers, proton pump inhibitors, synthetic prostaglandins, or GI protectants can be used to treat ulcers, none are effective prophylactically.

Blood transfusions may be required in some cases. The decision whether to transfuse or not should be made based upon clinical signs rather than a PCV alone. If the patient is tachycardic, tachypnoeic, or very weak transfusion is indicated. It can be difficult to interpret cross match results if there is autoagglutination. Transfusion of DEA 1 negative red cells is an acceptable option. It must be remembered that the transfused cells may have a short half-life in this disease. If available, haemoglobin products (such as oxyglobin) are an alternative to red blood cells.

These products will result in interference with biochemical testing, but have the advantage that they cannot be haemolysed.

There is much debate about whether stronger immunosuppressive agents are required routinely to treat IMHA (and other immune mediated diseases). Some feel that they are not necessary in all cases. I tend to use them early on routinely, to try and gain control of the disease as soon as is possible, and also for their glucocorticoid sparing effects. They can also be used in severe cases, indicated by a very low PCV, severe clinical signs, autoagglutination, intravascular haemolysis, or the need for repeated transfusions. There are a number of drugs that can be used, including azathioprine, cyclophosphamide, or cyclosporine.

Azathioprine is a Purine antagonist that acts on T cells. By inhibiting T helper cells it can modify the humoral response. The dose rate is 50 mg/m^2, or 1-2 mg/kg every 24 –48 hours. It can take 14 days or more to start seeing the effects of azathioprine, which s one of the justifications for initiating therapy early on. Side effects are not common, but include potential myelosuppression, hepatotoxicity, nephrotoxicity and pancreatitis. Blood counts should be periodically monitored.

Cyclosporine is a powerful T cell immunosuppressive that has its effect via the inhibition of Interleukin 2. It is recommended to use the newer microemulsion forms that have more reliable gastrointestinal absorption. Blood should be collected for therapeutic drug monitoring after a few days, with the dose adjusted accordingly. Side effects can include gingival hyperplasia, gastrointestinal upset, hepatotoxicity and nephrotoxicity. There have been suggestions of an increased risk of lymphosarcoma with prolonged use. The drug is also expensive, but can be used early on to gain control of the disease, and can then be replaced by azathioprine in the long term.

Cyclophosphamide is an alkylating agent that has effects on both T and B cells. It has a rapid onset of action, and can be administered orally or intravenously. The dose rate is 200 mg/m^2 every 7 days, or 50 mg/m^2 every second day or 4 times per week. Side effects include myelosuppression, gastrointestinal side effects, and sterile haemorrhagic cystitis. The latter can be minimised by administering the drug of a morning, and maximising water intake and opportunities to urinate.

Leflunomide is an inhibitor of pyrimidine biosynthesis. It has been used in dogs to prevent transplant rejection, and has promise in the therapy of IMHA. The starting dose is 4 mg/kg daily.

Other therapies include the use of human intravenous gamma globulin (to try and bind immunoglobulin), or plasmapheresis. Some advocate splenectomy to control haemolysis, but the results are variable.

Monitoring the response to treatment is also important. Serial PCV's and TP's should be performed, with the frequency determined by the severity of disease. Early on a stable PCV may indicate a balance between red cell production and lysis. The drugs should be continued until the PCV is normal. The length of time before this occurs is variable. Once stable the glucocorticoids can be slowly tapered – no more than a 20 - 25% reduction of the current dose ever 2 weeks if the patient is stable. If relapse occurs as the dose is tapered, they will need to be reinstated at the higher dose rate until the disease is again controlled. A decision can be made whether to taper the glucocorticoids to a minimal dose (or stop them) while maintaining the other immunosuppressive drug, or whether to use the glucocorticoids alone as the maintenance drug. I tend to taper the glucocorticoids and maintain the azathioprine, which can be slowly tapered. Some patients will need a maintenance level of drug in the long term.

The long-term prognosis is variable in IMHA. In some long-term studies at referral institutions the one-year survival is as low as 30%. This may be influenced by the nature of cases seen at referral hospitals. In some patients the IMHA cannot be controlled despite aggressive therapy. In other cases the drugs cannot be tapered because of relapses. In some patients the side effects of the therapies (e.g glucocorticoid related PU/PD) are the reason an owner will euthanase their pet.

Thromboembolic disease is a potential complication of IMHA and its therapy. IMHA is itself a risk factor for thromboembolism, especially in patients with autoagglutination. This is exacerbated by the use of glucocorticoids, which can make the patient hypercoagulable. Patients that are icteric, have intravenous catheters, or receive blood transfusions are at even higher risk. Pulmonary thromboembolism is the most common thromboembolic side effect. This may manifest as tachypnoea, dyspnoea, exercise intolerance or cyanosis. Thoracic radiographs may be unremarkable in the acute phase, or may show pulmonary vascular changes (enlarged arteries), parenchymal disease, or small volume pleural effusion. Arterial blood gas analysis is helpful to determine the severity of disease. In PTE there will be hypoxaemia and hypocapnia, the latter in response to hyperventilation. Heparin can be used prophylactically, or if PTE is occurring to try and prevent further thromboembolic change. The dose rates and efficacy of heparin are not well defined.

Non Regenerative Anaemias

Non regenerative anaemias may be related to primary bone marrow disease, or a disease outside of the marrow that reduces erythropoiesis.

Diseases of the Bone Marrow

There are a number of drugs that have the potential to result in altered erythropoiesis, either as an isolated event or as part of a pancytopaenia. These include many antineoplastic drugs, not limited to the alkylating agents (e.g. cyclophosphamide), the vinca alkaloids, platinum drugs, hydroxyurea, or dacarbazine. Typically because of their shorter lifespan, the effects will be seen earlier in the neutrophils and platelets. Whilst any drug can potentially cause anaemia, ones more commonly implicated include phenylbutazone, phenobarbitone, chloramphenicol, trimethoprim-sulphonamide combinations, fenbendazole, quinidine, and some cytotoxic drugs such as azathioprine. Radiation can also cause cytopaenias.

Oestrogen, either endogenous (ovarian disorders in females, sertoli cell neoplasia in males) or exogenous may result in pancytopaenia. The mechanisms implicated include reduced haematopoietic stem cell numbers, inhibition of stem cell differentiation, and possibly an altered response to EPO. The most common initial finding is a neutrophilia which may persist for some weeks, but a pancytopaenia will typically develop as a result of bone marrow hypoplasia. Removal of the source of oestrogen may not result in reversal of the bone marrow hypoplasia.

Infectious agents have the potential to result in anaemia through damage to erythroid precursors, or damage to the bone marrow stromal cells. Parvovirus can proliferate in progenitor and proliferative cells in the bone marrow. Marrow examination may show degenerative changes in the haemopoietic cells, and possibly an increased number of phagocytic macrophages. Ehrlichia infections can cause a bone marrow hypoplasia in the chronic phase of infection. A blood count will typically reveal a pancytopaenia. Bone marrow examination will reveal marrow hypoplasia, often with increased numbers of plasma cells.

Whilst immune mediated disease is more typically associated with haemolysis, in some patients a non regenerative anaemia may be the result.. This may be because the immune response is directed at the reticulocytes, or the attack may occur in the precursor cell compartment, the latter being referred to as ‘Pure red cell aplasia’. The white cells and platelets are typically spared. The anaemia may be severe in some cases. A bone marrow aspirate may show a maturation arrest, with presence of the early erythroid series (or even expansion) and the absence of later series if the immune response is directed against marrow cells, or there may be erythroid hyperplasia if the reticulocytes are the target. Treatment is as described for immune mediated haemolytic anaemia. The prognosis is variable, and not all patients will respond to therapy.

Myelophthisis refers to the replacement of bone marrow by non marrow elements. This may be neoplastic cells, granulomas, fibrous tissue and lipid storage cells. Anaemia used to be considered to be a ‘crowding-out’ phenomenon in the past in such situations. It is more likely that there is competition between the normal marrow elements and invading cells for nutrients, and that the invading cells likely produce cytokines that suppress the haemopoietic cell lines.

Haemopoietic neoplasms commonly cause a non regenerative anaemia. There may or may not be an increased number of cells of the neoplastic line in the peripheral blood. Other haemopoietic and lymphoid tissues may become infiltrated, including lymph nodes, spleen, and liver. Lymphoproliferative neoplasia is more common than myeloproliferative disorders in dogs. Affected patients may show systemic signs of disease, including fever, lethargy, inappetence, or gastrointestinal signs. Bone pain may sometimes be noted upon examination, along with lymph node enlargement or organomegaly. Haematology may show signs of leukaemia in addition to anaemia. Biochemical profiles may reflect organ damage. Cytology of bone marrow or node or organ aspirates may reveal neoplastic cells. In some situations bone marrow core biopsy will be necessary. In many cases morphology of the neoplastic cells alone may not provide an answer as to the cell line of origin. There are newer immunophenotyping techniques, which use specific monoclonal antibodies to differentiate antigens. Flow cytometry has become very important in identifying the cause of leukaemias. Therapy can be more challenging in these cases. Some lymphoid neoplasms and multiple myeloma may show some response to chemotherapy, but this is variable. Myeloproliferative diseases tend to be less responsive.

Myelofibrosis can be associated with neoplastic diseases of the marrow, or with chronic stimulation of the bone marrow with chronic haemolytic anaemia, such as seen with pyruvate kinase deficiency. Idiopathic myelofibrosis may be associated with a megakaryocytic haemopoietic stem cell disorder. Affected patients typically have a non-regenerative anaemia. There may be evidence of extramedullary haemopoiesis. Bone marrow aspiration is difficult, and typically a core biopsy must be collected.

Myelodysplastic syndromes are characterised by cytological dysplasia of one or more of the cellular elements in the bone marrow or blood. The results may include anaemia, neutropaenia or thrombocytopaenia, or various combinations of these cytopaenias. Dysplastic progenitor cells proliferate rapidly, but typically fail to differentiate or mature normally. The condition may be secondary to drugs (chemotherapy drugs, dilantin) or toxins (radiation, lead), or deficiency disorders (iron, copper, folate, vitamin B12), or immune mediated disease. The condition may represent a preleukaemic state. It may also be congenital, such as Vitamin B12 malabsorption in Giant Schnauzers. This disease is characterised by chronic non regenerative anaemia and neutropaenia, plus morphological changes including microcytes, macrocytes, acanthocytes, schizocytes, elliptocytes and hypersegmented neutrophils.

Extramarrow Diseases

Renal Failure

The anaemia associated with chronic renal failure is multifactorial in nature. The reduced number of functional nephrons can result in a decrease in EPO production. However this is certainly not the sole cause for the anaemia, as studies examining bone marrow aspirates on affected patients show variable marrow cellularity, but many have normal cellularity, myeloid to erythroid ratios and erythroid development. Other toxins may contribute to reduced red cell production. Other contributing factors include reduced erythrocyte lifespan, reduced nephron sensitivity to hypoxaemia, reduced platelet function, inappetence reducing nutrient intake, increased parathyroid hormone concentrations suppressing erythropoiesis, and gastrointestinal blood loss.

Therapy to combat anaemia in renal failure can be multifactorial. Steps to maintain food intake can be important, as well as pharmacologic therapy to reduce gastrointestinal blood loss (H2 blockers, proton pump inhibitors). Erythropoietin therapy may be of benefit in patients that are showing clinical signs of their anaemia. When using the human recombinant EPO products antibody production will limit the duration of use, with antibodies affecting both exogenous and endogenous EPO. The starting dose of EPO is 100 units/kg three times a week S/C, and the dose is tapered as the target PCV is reached (often 25% -30% is adequate). Iron supplementation should be provided to patients receiving EPO. If the PCV begins to fall on therapy, antibody production is likely, and therapy will need to be discontinued. At Cornell University in New York State a recombinant canine EPO has been developed. Transfusion therapy can provide shorter term relief to patients with renal failure associated anaemia.

Anaemia of Inflammatory Disease

Anaemia of inflammatory disease may be associated with acute or chronic inflammatory diseases, with possible causes including trauma, infection, immune mediated disease or neoplasia. It is a common cause of anaemia in practice. The anaemia tends to be mild in dogs, but may become severe in cats.

Pathophysiologically the disease is likely caused by cytokine production. The result can be iron sequestration, reduced red cell lifespan, and reduced erythropoiesis. The aim of the iron sequestration is thought to be an attempt to deprive infectious agents of iron that may be necessary for their metabolism. Apolactoferrin is found in milk, neutrophils and some mucosal secretions, and it can bind large amounts of iron, and this is increased at the lower pH associated with inflammation. Interleukin 1 and tumour necrosis factor alpha increase the synthesis and release of apolactoferrin, and neutrophils release it at the site of inflammation, and macrophages express increased lactoferrin surface receptors, and they can internalise lactoferrin bound iron. Iron is diverted to slow release pathways (haemosiderin). Intestinal epithelial cells may also be affected, reducing iron absorption.

The anaemia is characterised by reduced EPO production, reduced marrow response, and reduced erythropoiesis (iron limited). The anaemia will be normocytic-normochromic. Iron studies will show normal reduced serum iron and total iron binding capacity, and possibly increased marrow iron stores, and increased ferritin. Therapy is related to the correction of the underlying disease.

Anaemia related to Endocrine and Metabolic Disease

The endocrinopathies that can be associated with non regenerative anaemia include hypothyroidism and hypoadrenocorticism. In hypothyroidism the anaemia is mild, and may not be contributing to the clinical signs. The cause is due to reduced erythropoietin production associated with reduced oxygen consumption. The diagnosis may be supported by a mild non regenerative anaemia and biochemical changes such as hypercholesterolaemia. The diagnosis can be confirmed using hormonal studies (total T4, free T4, TSH concentration, antithyroglobulin antibodies), or imaging studies. In hypoadrenocorticism the anaemia can be due to marrow hypoplasia because of the hypocortisolaemia (cortisol may have a permissive action in the bone marrow). Gastrointestinal blood loss may cause a more severe anaemia. Haematology may show a non regenerative anaemia and the lack of a stress leucogram. Biochemistry may show azotaemia and electrolyte abnormalities. The diagnosis can be confirmed by assessing the response to ACTH.

Transfusion Therapy in Non Regenerative Anaemia

Due to the slow, insidious onset of anaemia with many non regenerative causes of anaemia, clinical signs of the anaemia itself may not be apparent until the PCV is quite low. Transfusions are rarely required for more moderate anaemias, or patients with anaemia of inflammatory disease. Some patients with pure red cell aplasia may require transfusions while waiting for therapy to take effect. Other times transfusion may be undertaken in patients with more severe marrow disease to try and improve short term quality of life.

If patients may be in a situation to require multiple transfusions it is ideal to cross match first, or have an idea of blood groups. The ideal donor is a DEA 1 negative dog.

In patients that are not hypovolaemic the use of packed red blood cells is preferable to avoid volume expansion. A transfusion of 1ml/kg of packed red blood cells will typically increase the PCV by approximately 1%. Blood obtained from a blood bank such as the University of Melbourne has the advantage of DEA 1 negative donors, and donors that have been tested for potential blood borne pathogens.

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ANAEMIA CASE STUDIES

Case: **“Chester”**

Signalment: Four year old male Cavalier King Charles Spaniel

Presenting Complaint: Collapse

History: Chester’s owners had been away on two weeks holiday, and a neighbour was feeding Chester. He was an outside dog. When the owners arrived home today they found him in a collapsed state. He was normal before they went away.

Physical Examination:

Temp = 37.5

HR = PR = 154, pulse regular

RR = 40

MM = White

CRT = unable to detect

Chester was unable to stand. There was a large amount of flea dirt encrusted in his coat. There was tachypnoea. A Grade V/VI systolic heart murmur was noted, loudest on the left side. There were bilateral jugular pulses.

1. **What are Chester’s major problems?**

2. **What are the potential aetiologies?**

3. **What would be your diagnostic plans?**

4. **Would you undertake any immediate therapy?**

Complete Blood Count

WBC	34.99	(5.5-16.9)

Lymphocytes	1.52 x10^9/L	(0.5-4.9)
Monocytes	2.58 x10^9/L	(0.3-2.0)
Neutrophils	30.27 x10^9/L	(2.0-12.0)
Eosinophils	0.56 x10^9/L	(0.1-1.49)
Basophils	0.07 x10^9/L	(0.0-0.1)

%Lymphocytes	4.3%
%Monocytes	7.4%
%Neutrophils	86.5%
%Eosinophils	1.6%
%Basophils	0.2%

Hct	8.3%	(37-55)
RBC	1.3 x 10^12/L	(5.5-8.5)
Hb	3.3 g/dl	(12.0-18.0)
Retic	64.8 X 10^3/uL	
%Retic	5.2 %	
MCV	65.9 fL	(60.0-77.0)
RDW	20.7%	(14.7-17.9)
MCHC	39.3 g/dL	(30.0-37.5)
MCH	25.90 pg	(18.5-30.0)
Plt	184 x 10^3/uL	(175-500)
MPV	-- fL	
PCT	0.7%	
PDW	26.3%	

Biochemistry

Na	147. mmol/l	(144-160)
K	4.6 mmol/l	(3.8-5.8)
Cl	119. mmol/l	(93-122)
Bicarb	13.8 mmol/l	(14-28)
Anion Gap	19. mmol/l	(15-25)
Urea	9.3 mmol/l	(1.7-6.5)
Creatinine	45. umol/l	(40-140)
Glucose	5.8 mmol/l	(3.5-6.7)
Bilirubin	1.7 umol/l	(3.0-22.0)
AST	61. U/l	(1-80)
ALT	223. U/l	(3-70)
AlkPhos	82. U/l	(20-155)
T Protein	55. g/l	(53-75)
Albumin	27. g/L	(23-39)

Globulin	28. g/l	(31-39)
Calcium	2.35 mmol/l	(2.0-2.8)
Phosphate	1.41 mmol/l	(1.3-2.0)
CreatKinase	89. U/l	(0-180)
Amylase	577. U/l	(1-2400)
Lipase	1270. U/l	(250-1750)
Cholesterol	3.17 mmol/l	(3.6-8.8)

Case: “Celtic”

Signalment: Five Year Old Male Irish Wolfhound

Presenting Complaint: Lethargy

History: The owners noted that “Celtic” had been obtunded and lethargic for a few days. The clinical signs had been progressive in nature, so he was presented to the emergency service.

Physical Examination: Weight 53.6 kg, Temp = 101.4 degrees, HR = PR = 80, RR = 48, MM = Pale but icetric, CRT = 2 sec. There was icterus of the membranes, sclera and pinnae. He was obtunded, and was reluctant to stand. Splenomegaly was apparent on abdominal palpation.

1. **What do you think are the high yield problems for this patient?**

2. **If you could only perform one test to begin with, what would it be?**

3. **What are your diagnostic and immediate therapeutic plans for this patient?**

CBC

WBC/µl	**47,520**	(5800-11700)
Band	0	(<300)
Seg	**35,640**	(3000-7100)
Lymph	**849**	(1100-5100)
Mono	**5,940**	(<800)
Eos	0	(<100)
Baso	0	(0)
RBC, X 10^6/µl	*	(5.6-8.5)
Hb, g/dl	*	(13-19)
PCV, %	**16**	(38-59)
Platelets/µl	162,000	(157-394 X 10^3)
MCV, fl	*	(64-73)
MCHC, g/dl	*	(33-37)

* Unable to read because of agglutination in tube

SERUM CHEMISTRIES

Urea, mmol/L	7.0	(1.7-6.5)
Creatinine, umol/L	***icterus**	(40-140)
Glucose, mmol/L	6.0	(3.6-6.7)
ALT, IU/L	**110**	(21-67)
AP, IU/L	**455**	(14-72)
Chol, mmol/L	8.3	(3.6-8.8)
T. Prot, g/L	58	(56-76)
Albumin, g/L	30	(25-38)
Globulin, g/L	28	(27-38)
Calcium, mmol/L	2.5	(2.0-2.8)
Phos, mmol/L	1.50	(1.3-2.0)
Sodium, mmol/L	**146**	(147-157)
Potassium, mmol/L	**3.4**	(4.4-5.3)
Chloride, mmol/L	114	(111-122)
TCO_2, mmol/L	18	(18-24)

URINALYSIS

Collection	cystocentesis
USG	1.017
pH	7.0
Protein	+
Glucose	neg
Ketones	neg
Bilirubin	+++
Blood	++

Sediment 0-2 RBC/hpf, 0-2 WBC/hpf, fat droplets

Case: “Belle”

Signalment: Eight year old female spayed American Staffordshire Terrier

Presenting Complaint: Anaemia

History: Belle had parvovirus infection as a puppy (this is how she was obtained by her current owner who was the attending veterinarian) and then when three years old she developed immune mediated thrombocytopaenia that responded to prednsiolone. Six months prior to presentation Belle developed hind-limb ataxia and weakness that was responsive to prednisolone. It was noticed that she had some patchy skin discoluration a few weeks prior to presentation. Blood was collected – her PCV was 33%, but the rest of the haemogram was normal. A biochemical profile showed elevated lipase at 86 u/L (<70), hypertriglyceridaemia and an elevated albumin (43 g/L). The skin lesions responded to Cephalexin, but her PCV remained around 30%.

Physical Examination:

Temp = 38.1

HR = PR = 100, pulse regular

RR = 28

MM = Pink

CRT = 1 sec

Belle was alert upon examination. She had a stiff hind limb gait with muscle atrophy and some mild ataxia. She had some small cutaneous nodules. There were some pustules on her abdomen. She had mild dental tartar. Abdominal palpation revealed hepatomegaly.

1. **What are Belle’s high yield problems?**

2. **What would be on your list of differentials?**

3. **What are your diagnostic plans?**

Urinalysis

Volume: 2.0 ml
Colour: yellow
Transparency: transparent
Urine Spec Gravity: 1.040
Glucose: Negative
Bilirubin: Negative
Ketones: Negative
Hemoglobin: Negative
pH: 6.5
Protein: +
Urobilinogen: Normal (less than 20umol/L
RBC/hpf: 0.0
WBC/hpf: 0.0
Casts: None
Bacteria: Negative
Fat: Negative

Epith Type: None
Sperm: Negative
Crystals: None

Debris: few
Comments: Trace protein is not significant.

CBC

Hb	116 g/L	(115-180)
RCC	4.6 x 10^12/L	(5.0-8.0)
Hct	0.33	(0.37-0.55)
MCV	72 fl	(63-74)
MCH	25 pg	(20-25)
MCHC	351 g/L	(310-360)
Platelets 217		(200-900)
WBC	8.6 x10^9/L	(6.0-14.0)
Neuts	5.4 x10^9/L	(4.1-9.4)
Lymph	2.6 x10^9/L	(0.9-3.6)
Mono	0.3 x10^9/L	(0.2-1.0)
Eos	0.3 x10^9/L	(0.1-1.2)
Baso	0.0 x10^9/L	(<0.1)
Retics	0.9 %	
Retics abs41		

Red cells	anisocytosis +, occasional polychromasia
White cells	normal
Platelets	normal

Case: “Rocky”

Signalment: Four year old MN Shih Tzu

Presenting Complaint: Weakness

History: Rocky had been diagnosed with hypoadrenocorticism eleven months previously, and had been well managed with Cortisone 5mg every 12 hours and Fludrocortisone 0.1 mg every 12 hours. Ten days prior to presentation he had received C4 and rabies vaccines as he was about to move overseas. Within 2 days he had become inappetent and had become progressively lethargic.

Physical Examination:

Temp = 39.4

HR = PR = 148, pulse synchronous

RR = 28

MM = Pale

CRT = 1.5 sec

Rocky was subdued but responsive upon examination. He had mild dental tartar. There was a Grade II/VI plateau type systolic murmur at the left heart base. Abdominal palpation demonstrated splenomegaly.

CBC

Hb	42 g/L	(115-180)
RCC	2.0 x 10^12/L	(5.0-8.0)
Hct	0.13	(0.37-0.55)
MCV	62 fl	(63-74)
MCH	21 pg	(20-25)
MCHC	333 g/L	(310-360)
Platelets	270	(200-900)
WBC	8.7 x10^9/L	(6.0-14.0)
Neuts	5.2 x10^9/L	(4.1-9.4)
Lymph	2.6 x10^9/L	(0.9-3.6)
Mono	0.4 x10^9/L	(0.2-1.0)
Eos	0.4 x10^9/L	(0.1-1.2)
Baso	0.0 x10^9/L	(<0.1)

Red cells	Anisocytosis +, Polychromasia +, Microcytosis +. Occasional nucleated red blood cell, Spherocytes.
White cells	Occasional band forms
Platelets	Normal

Biochemistry

Sodium	136 mmol/L	(144-154)
Potassium	4.9 mmol/L	(3.8-5.8)
Chloride	104 mmol/L	(93-121)
Bicarbonate	22 mmol/L	(14-28)
Anion Gap	15 mmol/L	
Urea	4.9 mmol/L	(1.7-6.5)
Creatinine	0.07 mmol/L	(0.04-0.14)
Plasma Gluc	5.6 mmol/L	(2.8-6.7)
Glucose	5.3 mmol/L	(2.8-6.7)
Bilirubin	4 umol/L	(<10)
AST	20 U/L	(1-80)
ALT	13 U/L	(1-70)
GGT	15 U/L	(<5)
Alk Phos	28 U/L	(1-120)
Protein	66 g/L	(53-75)
Albumin	36 g/L	(22-36)
Globulin	30 g/L	(31-39)
Alb:Glob	1.2	
Calcium	2.58 mmol/L	(2.00-2.80)
Phosphate	1.14 mmol/L	(1.30-2.00)
Creatine Kinase	108 U/L	(<180)
Cholesterol	4.5 mmol/L	(3.6-8.8)
Triglyceride	2.5 mmol/L	(0.1-0.4)

Case: “Brew”

Signalment: Five year old MN Labrador Retriever

Presenting Complaint: Quiet

History: Brew had been presented with a left hind-limb lameness two weeks ago, and was found to have a cruciate rupture. Pre surgical blood work showed a PCV of 35% (no reticulocyte count was completed), and a platelet count of 100,000/uL with clumps reported. The surgery proceeded without complication. The owner (a vet student) had noted he had been quiet post surgery. He had represented for suture removal, and the surgeons were concerned that he was pale, so the medicine service was asked to examine the patient.

Physical Examination:

Temp = 39.5

HR = PR = 140, pulse regular

RR = 36

MM = Pale pink

CRT = 1 sec

Brew was quiet upon examination. His lymph nodes were of normal size but firm. Thoracic auscultation was unremarkable. Abdominal palpation revealed splenomegaly. His left hind limb was shaved, and he had a mild lameness.

1. **What are Brew’s high yield problems?**

2. **What are the most likely causes for these signs?**

3. **What are your initial plans – diagnostic or therapeutic?**

Complete Blood Count

RBC		3.8	(4.9 - 8.2) X10 ^12 /L Low
Haemoglobin		92	(100 – 206) g/L Low
Haematocrit		0.26	(0.35 - 0.58) L/L Low
MCV		68	(64 – 76) fL
MCH		24	(21 – 26) pg
MCHC	354	(310 – 360) g/L	
Platelet Count		81	(200 – 500) X10 ^9 /L Low
Reticulocyte Count			(0.3 0 - 1.6) %
Reticulocyte Abs		11	(0 – 81) X10 ^9 /L
WCC		10.7	(4.5 - 17.0) X10 ^9 /L
Neutrophil %		81 %	
Neutrophil		8.7	(3.5 - 12.0) X10 ^9 /L
Lymphocyte %	10 %		
Lymphocyte		1.1	(0.9 - 3.5) X10 ^9 /L
Monocyte %		9 %	
Monocyte		1.0	(0 - 1.2) X10 ^9 /L
Eosinophil %		0 %	
Eosinophil		< 0.1	(0 - 1.5) X10 ^9 /L
Basophil %		0 %	
Basophil			< 0.1 X10 ^9 /L
Plasma Protein	75	(62 – 85) g/L	

Biochemistry

Na	149 mmol/l	(144-160)
K	4.0 mmol/l	(3.8-5.8)
Cl	114 mmol/l	(93-122)
Bicarb	18.0 mmol/l	(14-28)
Anion Gap	21 mmol/l	(15-25)
Urea	6.0 mmol/l	(1.7-6.5)
Creatinine	130 umol/l	(40-140)
Glucose	5.1 mmol/l	(3.5-6.7)
Bilirubin	9.9 umol/l	(3.0-22.0)
AST	52 U/l	(1-80)
ALT	140 U/l	(3-70)
AlkPhos	240 U/l	(20-155)
T Protein	68 g/l	(53-75)
Albumin	23 g/L	(23-39)
Globulin	45 g/l	(31-39)
Calcium	2.19 mmol/l	(2.0-2.8)
Phosphate	1.48 mmol/l	(1.3-2.0)
CreatKinase	161 U/l	(0-180)
Amylase	1473 U/l	(1-2400)
Lipase	677 U/l	(250-1750)
Cholesterol	5.97 mmol/l	(3.6-8.8)

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